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VARIATION IN WATER, SODIUM AND POTASSIUM CONTENT OF HUMAN SKELETAL MUSCLE AND THEIR RELATIONSHIP TO AGE AND SEX

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR

THE DEGREE OF MASTER OF SCIENCE

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BY

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UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Variation in Water, Sodium and Potassium Content of Human Skeletal Muscle and their Relationship to Age and Sex" submitted by Teh-chang Shih in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The literature concerned with previous investigation into human skeletal muscle electrolyte is briefly reviewed. It was found that some investigators have studied muscle electrolyte content by analysis of living human muscle tissue and the number of samples of normal and diseased tissue has been small.

The objective of this study was the systematic evaluation of water, sodium and potassium content of normal living striated muscle and to study the use of muscle biopsy in certain disease states which might be expected to have associated muscle electrolyte abnormalities permitting an evaluation of its clinical usefulness.

Skeletal muscle obtained from 89 adults (131 samples), 17 children (17 samples) and 8 patients with disturbance of cardiac, renal or muscle function were analyzed for water, sodium and potassium using the chemical and flame photometric methods of Litchfield and Gaddie.

The results of muscle composition from normal subjects are essentially in agreement with those published by previous investigators.

Different muscle groups vary in sodium and potassium content and these variations are noted in samples from different muscles obtained from the same subject.

A negative correlation between sodium and potassium content is demonstrated when expressed with fat free wet weight or fat free dry weight and this correlation may not be present in the muscle samples with a great variation of water content in terms of

Fat free dry weight. A positive correlation between the muscle content of water and the sum of sodium plus potassium content is observed when expressed as fat free dry weight.

A significant difference in potassium, sodium and water content in the muscle of children as compared with adults is noted, the potassium being lower and the sodium and water being higher in children.

Muscle biopsies were obtained from the site of the pectoralis major of 18 females, age 18 to 86 years. It was found that water and sodium content in the muscle of the older age group was higher than those of the younger age group when expressed as fat free dry weight.

A significant difference in water content of female muscle from that in male muscle is found, the water being higher in the female.

Muscle sample size and muscle edema in patients with disturbance of cardiac, renal or muscle function are discussed.

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I. INTRODUCTION

In recent years a number of papers concerning human skeletal muscle electrolyte have been published, (4, 5, 8, 14, 16, 24). The muscle biopsy has often been used in studies of metabolic disorders (e.g. striated muscle in acidosis and alkalosis by Mudge et al. (51) in 1949) and diseases of the endocrine glands (e.g. the muscle potassium in thyrotoxicosis by Staffurth et al. (61) in 1965, and water and electrolyte content of skeletal muscle in acromegaly by Palmieri et al. (54) in 1965), cardiac disease (e.g. potassium deficiency in congestive heart failure by Cort (13) in 1954) and for clinical application (e.g. muscle biopsy for clinical research on body composition by Muldowney et al. (52) in 1963). The results of many studies were obtained by the examination of post-mortem tissue, (14, 16, 25, 35, 45). Death may have produced secondary chemical derangements. For instance, in Iseri's series (35) in 1952, eleven pectoralis major muscle biopsies (control group) obtained from autopsy material showed a great variation in electrolyte composition when compared with other series in which muscle biopsy was obtained from living tissue (8, 66, 67). The results of those series are summarized in Appendix Table 2.

Some investigators have studied muscle electrolyte content by analysis of living human muscle tissue; however, the number of samples of normal and diseased tissue has been small. The largest series published contains no more than fifty cases. A systematic investigation of living human muscle electrolyte content and its relationship to sex and age has not been reported.

Muldowney et al. in 1963 (52) on a study of eight cases of disturbance of serum sodium concentration found that inadequacy of bed-

side clinical assessment of body water and sodium in stressed apparent "dehydration" may co-exist with genuine water depletion or with gross excess of body water or sodium. They reported the muscle analysis provides a useful index of overhydration or underhydration.

The objective of this study was the systematic evaluation of water, sodium and potassium content of normal living striated muscle, and to study the use of muscle biopsy in certain disease states which might be expected to have associated muscle electrolyte abnormalities permitting an evaluation of its clinical usefulness.

In the comparison of muscle values of normal subjects published by various authors (see Appendix Table 2) the values of water and electrolyte content in the muscle vary from series to series. The use of various anesthetic agents may be one of the factors causing these differences. The influence of general anesthesia and preoperative medication on cell electrolyte content has been reported by Moore et al. (48) in 1960 and other investigators (15, 30). Therefore, ideally the normal muscle electrolyte value should be established by the analysis of muscle obtained directly from unanesthetized normal subjects. On the other hand, the numbers of normal subjects who would submit to muscle biopsy was found to be too small to permit a useful study. The possible error of water and electrolyte content introduced by general anesthesia was accepted for the purposes of this study.

In the study to be reported human living skeletal muscle was biopsied and analyzed on 114 subjects undergoing general anesthesia for elective surgical conditions. Of the 114 cases, eight were patients with muscle wasting, cardiac, or renal disease. In the remaining patients, no alteration of water, electrolyte or muscle metabolism was

was expected. A variation in muscle electrolyte composition possibly related to age, sex and site of muscle has been studied.

II. MATERIALS AND ANALYTICAL METHODS

(A) MATERIALS

From 89 adults and 17 children undergoing surgical procedures under general anesthesia, 131 adult muscle and 17 children muscle biopsies were obtained. Most biopsies were taken within 20 minutes of the induction of the anesthesia. Samples from the pectoralis major and pectoralis minor were taken at intervals greater than 20 minutes due to the longer surgical exposure time. These biopsies were frequently obtained later than 1 1/2 hours following the induction of anesthesia. None of the patients biopsied suffered renal insufficiency, caridac decompensation, muscle wasting diseases or any recognized clinical disease that would be expected to disturb water and electrolyte metabolism. The diagnoses and the muscle biopsies obtained are tabulated in Table 1 for adult patients and in Table 2 for children. For contrast and interest, a further eight muscle biopsies were obtained from patients with muscle wasting,cardiac or renal disease.

(B) ANALYTICAL METHODS

The analytical procedure described by Litchfield and Gaddie for the determination of muscle sodium and potassium employing an Eel Flame Photometer was used (39). Excess blood was removed gently by blotting the muscle on filter paper and visible connective tissue and fat were removed mechanically. The biopsy specimen was then divided into two equal parts, with the resulting average weight of each portion

TABLE 1

DISEASE OF ADULT PATIENTS AND MUSCLE BIOPSIES OBTAINED

	Cases		Sites	
	F	M	Female	Male
Elective orthopaedic procedure	15	24	VL 8 ES 3 VI 3 TFL 2 GMa 1 VM 1 GMe 1 RF 1 Sa 1	ES 6 De 5 Gma 2 GMe 1 VL 6 Il 1 TFL 3 VM 1 VI 2 RF 1 TP 1 AL 1 AB 1 Sa 1
Elective gastro-intestinal lesion (Including disease of gall bladder)	6	5	RA 3 Inc 2 SPI 1 ES 1 LD 1 RM 1 SA 1	RA 3 EO 1 SA 1
Benign lesion of genital tract	11	0	RA 10 PY 1	
Malignant lesion of genital tract	4	0	RA 4	
Benign lesion of lung	1	1	SA 1 Inc 1 LD 1	SA 1 LD 1 Inc 1 RM 1
Malignant lesion of lung	0	2		Inc 2 SA 1 LD 2
Breast disease	20	0	PMa 18 PMi 16 LD 1 SA 1	
TOTAL	57	32	85	46
	89		131	

Symbols:

Pectoralis major = PMa Pectoralis minor = PMi Vastus lateralis = VL
Deltoid = De Rectus abdominis = RA Vastus intermedius = VI
Erector spinae = ES Intercostal = Inc Serratus anterior = SA
Tensor fasciae latae = TFL Latissimus dorsi = LD Rhomboid major = RM
Gluteus medius = GMe Vastus medialis = VM Rectus femoris = RF
Gluteus maximus = GMa Iliacus = Il Serratus post. inf. = SPI
Adductor longus = AL Ext. obliq. abd. = EO Pyramidalis = Py
Adductor brevis = AB Tibialis posterior = TP Sartorius = Sa

TABLE 2

DISEASE OF CHILDREN AND MUSCLE BIOPSIES OBTAINED

	Cases		Sites	
	F	M	Female	Male
Pectus carinatum	0	1		Pectoralis major 1
Congenital dislocation of hip	3	1	Vastus lateralis 2 Tensor fasciae latae 1	Tensor fasciae latae 1
Slipped capital epipysis	0	1		Vastus lateralis 1
Osteomyelitis	0	1		Gluteus medius 1
Lipoma in groin	0	1		Sartorius 1
Patent ductus arteriosus	0	3		Pectoralis major 2 Serratus anterior 1
Congenital Kyphoscoliosis	3	0	Erector spinae 3	
Congenital Shortening of left leg	0	1		Vastus medialis 1
Congenital pseudoarthrosis	1	0	Tibialis posterior 1	
Bone cyst	0	1		Deltoid 1
TOTAL	7	10	7	10
	17		17	

being 0.14 to 0.16 g. Each portion was then placed in an airtight glass container and was weighed immediately. The initial weights were corrected for water loss due to evaporation by repeated re-weighing of the muscles. The resulting weights were plotted against time. Weight loss was found to be linear with respect to time and the initial weight of tissues was determined by extrapolation of this straight line to zero time. In the material contained within this presentation, the time of delay from the operative removal until final weighing was regularly less than ten minutes. The samples were then dried in a hot air oven in glass containers overnight. Drying was continued in an oven to constant weight determined by repeated re-weighing. Water content of muscle was calculated by comparison of wet weight and constant dry weight. The containers were then filled with diethyl ether and permitted to stand for four hours decanting the ether and adding fresh ether every two hours. Subsequently, the muscles were replaced in a drying oven for an additional thirty minutes and then reweighed. Neutral fat content was calculated by the weight difference resulting from the ether extraction. The muscles were then considered to be fat and water free. These specimens were digested by adding 0.2 ml of concentrated nitric acid for each 100 mg. of muscle and digesting in a boiling water bath for 30 minutes. After cooling, the volume was adjusted to 10 ml. with the deionized distilled water. The solutions were then analyzed for sodium and potassium content in an Evans Electroselenium Ltd. flame photometer. The results of sodium and potassium analyses were expressed as a mean value on the basis of fat free wet weight and fat free dry weight.

For the study of the analytical methods employed (methods of

Litchfield and Gaddie), eight samples removed from one human muscle biopsy were analyzed for sodium, potassium, water and fat. It was found that the variation of water, fat and electrolytes among these 8 small samples was small. The results are tabulated in Table 3 (A).

TABLE 3 (A)

DATA OF 8 MUSCLE SAMPLES OBTAINED FROM SAME MUSCLE BIOPSY

No. of Samples	Sample Weight g WW	Fat. g/ 100g WW	Water ml/ 100g FFWW	K mEq/ kg FFWW	Na mEq/ kg FFWW
	Mean±S.D.				
8	0.177 ± .030	8.0 ±2.5	79.4 ± 0.4	82.4 ± 2.0	51.1 ± 1.8

S.D. = Standard deviation.
WW = Wet weight.
FFWW = Fat free wet weight.

A muscle biopsy was divided into 8 small pieces. Varying amounts of concentrated nitric acid were added to these small samples after extraction of fat. The results of analysis are shown in Table 4 (A) and it was noted that the difference of electrolytes among the samples was small.

TABLE 4 (A)

ELECTROLYTE CONTENT OF MUSCLE EXTRACTED IN VARYING AMOUNTS OF CONCENTRATED NITRIC ACID

No. of Samples	Amount of Nitric Acid added	Sample weight g WW	K mEq/ kg FFWW	Na mEq/ kg FFWW
			Mean	
2	0.1 cc/100 mg.	0.164	103.6	25.0
2	0.1 cc/ 75 mg.	0.116	107.0	20.0
2	0.1 cc/ 50 mg.	0.148	106.5	21.8
2	0.1 cc/ 25 mg.	0.121	104.8	22.8

Another further 8 samples with varying periods of time of extraction of fat were studied. The fat content in the samples with six hours of ether extraction is very similar to that using four or two hours for extracting fat. The results are tabulated in Table 5 (A).

TABLE 5 (A)

DATA OF MUSCLE SAMPLES OBTAINED FROM SAME MUSCLE BIOPSY WITH VARYING PERIODS OF TIME FOR EXTRACTING FAT

No. of Samples	Extraction (hours)	Sample Weight g WW	Fat g/ 100g WW	H_2O ml/ 100g FFWW	K mEq/ kg FFWW	Na mEq/ kg FFWW
		Mean				
4	6	0.093	1.3	77.0	101.3	33.4
2	4	0.092	1.4	76.9	102.1	30.0
2	2	0.129	1.2	77.1	99.3	36.0
N = 8	Grand mean		1.30	77.0	100.99	33.17
	S.D.		0.13	0.19	2.66	3.54

Two larger samples of muscle, 0.46 and 0.47 g in weight were analyzed and placed in ether for 4 hours. The results of analysis still show that fat content in larger samples is similar to that of smaller slices. The data was shown in Table 6 (A).

In Table 6 (A) one may find the difference in sodium and potassium contents between the last two samples and the first six samples. The last two samples consisted of four and five small pieces of muscle. The multiple cuts could lose potassium on scissors and contaminate these small pieces of muscle with excess extracellular fluid; however, fat content in the last two samples is very nearly the same as that in others.

Therefore, it is believed that the period of 4 hours may be enough for complete extraction of fat from these small muscle samples. From this experimental data, the method of Litchfield and Gaddie has been shown to be reliable for analysis of fat, water, sodium and potassium in human striated muscle for the purposes of this study.

TABLE 6 (A)

DATA OF MUSCLE SAMPLES OBTAINED FROM SAME MUSCLE BIOPSY WITH VARYING SAMPLE SIZES AND VARYING PERIODS OF TIME FOR EXTRACTING FAT

No. of Samples	Extraction (hours)	Sample Weight g WW	Fat g /100g FFWW	H_2O ml 100g FFWW	K mEq /kg FFWW	Na mEq /kg FFWW
		Mean				
2	4	0.468	4.5	78.3	96.6	30.5
2	4	0.157	3.3	78.4	99.4	27.5
2	6	0.173	2.8	78.1	97.8	30.0
2	6	0.143	3.2	78.4	90.0	37.0
N = 8	Grand mean		3.45	78.30	95.95	31.25
	S.D.		0.98	0.24	4.00	4.75

In this study, no attempt was made to calculate intracellular ion content because of the difficulty of measurement of extracellular space variation between duplicate muscle biopsies (8, 21). The chloride space was not determined because of a lack of exclusion of this ion from intracellular locations (22). Further, the time and technical limitations of the program did not permit the additional steps.

III. RESULTS

The results in the Table----(A) or Figure----(A) are expressed with fat free wet weight (FFWW) as the basis of reference for potassium (mEq/kg FFWW), sodium (mEq/kg FFWW), and water (ml/100g FFWW) and wet weight (WW) as the basis of reference for fat (g/100g WW).

The results in the Table---(B) or Figure---(B) are expressed with fat free dry weight (FFDW) as basis of reference for potassium (mEq/kg FFDW), sodium (mEq/kg FFDW), and water (ml/100g FFDW).

(A) Average Composition of All Skeletal Muscles

131 samples were obtained from 89 adult subjects early in the course of operation. The statistical results of average composition of muscles such as mean ($\bar{x}$) and standard deviation (S.D.) are tabulated in the Tables 7 (A) and 7 (B).

Tables 7(A) and 7 (B) show that potassium and sodium content are variable among different muscle groups, especially between pectoralis major and intercostal muscles. 10 large groups of muscles (Table 8) calculated on analysis of variance (29) revealed a significant difference of sodium and potassium content among the muscle groups. The results are tabulated in Tables 8 (A) and 8 (B).

Different muscles taken from each subject have also been studied. Using paired observations analysis by t-test (29), the results tabulated in Tables 9 (A) and 9 (B) continue to show a difference in potassium and sodium content between the muscles of the intercostal and serratus anterior or latissimus dorsi.

TABLE 7 (A)

DATA OF MUSCLE BIOPSY OBTAINED FROM 89 NORMAL ADULTS

Muscles	No.	Ave. Age	Water ml/ 100g FFWW	K mEq/ kg FFWW	Na mEq/ kg FFWW	K+Na mEq/ kg FFWW	Fat g/ 100g WW
					$\bar{x}$ ± S.D.		
Pectoralis major	18	55	78.1±0.54	94.0± 4.33	32.6± 7.70	126.6±5.86	1.78±1.85
Pectoralis minor	16	58	78.2±0.61	95.4± 5.58	29.9± 6.94	125.4±4.93	1.61±1.25
Rectus abdominis	20	38	76.6±0.71	86.6± 7.74	39.3± 9.47	125.9±5.16	2.45±1.61
Vastus lateralis	14	46	78.0±0.89	91.4± 3.93	34.0± 5.07	125.4±2.93	2.26±1.66
Vastus intermedius	5	29	78.3±1.14	87.6± 8.58	37.8± 8.92	125.4±3.15	0.82±0.59
Erector spinae	10	40	77.7±1.03	86.1±10.07	36.9±10.04	123.0±4.00	3.86±3.52
Intercostal	6	52	77.9±0.79	78.0± 6.20	49.1± 8.04	127.1±2.93	4.12±1.04
Serratus anterior	6	50	77.1±0.90	89.7± 8.39	36.1±10.00	125.8±4.92	2.45±1.52
Deltoid	5	29	77.0±0.44	87.0± 8.69	39.2± 9.41	126.2±1.83	1.19±1.46
Latissimus dorsi	6	49	77.7±0.90	90.5± 4.62	35.6± 5.89	126.1±6.60	2.33±2.06
Tensor fasciae latae	5	37	77.7±0.90	89.7± 4.50	36.4± 5.04	125.7±3.51	6.24±4.76
Gluteus maximus	3	35	78.0	85.9	39.9	125.7	8.27
Gluteus medius	2	44	77.3	84.0	37.8	121.8	4.91
Vastus medialis	2	36	77.3	96.0	31.9	127.9	1.03
Rectus femoris	2	23	77.0	96.6	28.1	124.7	1.13
Rhomboid major	2	41	77.6	80.9	49.5	130.4	0.82
Adductor longus	1	64	79.2	82.9	38.0	120.9	2.90
Adductor brevis	1	64	79.2	96.3	27.5	123.8	3.41
Iliacus	1	31	77.2	97.6	30.8	128.4	1.42
Ext. oblig. abd.	1	68	77.1	82.5	38.0	120.5	9.44
Tibialis posterior	1	29	77.7	90.7	32.3	123.0	2.27
Serratus post inf.	1	60	78.1	97.5	28.1	125.6	5.00
Pyramidalis abd.	1	48	78.2	92.5	39.5	132.1	2.77
Sartorius	2	14	77.5	91.2	32.3	123.5	0.04
TOTAL	131	44	77.84±0.78	89.76 ± 7.72	35.88± 8.75	125.64±4.25	2.59±2.46

TABLE 7 (B)

DATA OF MUSCLE BIOPSY OBTAINED FROM 89 NORMAL ADULTS

Muscles	No.	Ave. Age	Water ml/ 100g FFDW	K mEq/ kg FFDW	Na mEq/ kg FFDW	K + Na mEq/ kg FFDW
			$\bar{x}$ ± S.D.			
Pectoralis major	18	55	355.6±11.49	428.1±23.31	148.9±36.88	577.0±34.22
Pectoralis minor	16	58	359.8±12.84	437.8±21.92	137.9±35.03	575.7±32.17
Rectus abdominis	20	38	346.9±15.14	386.3±31.03	176.0±45.89	562.3±32.06
Vastus lateralis	14	46	354.5±17.93	414.9±12.49	154.1±26.76	569.0±26.50
Vastus intermedius	5	29	361.6±24.06	403.4±33.62	176.8±50.08	580.2±32.83
Erector spinae	10	40	348.5±21.02	385.0±36.91	167.0±51.47	552.0±33.34
Intercostal	6	52	352.7±16.14	352.7±25.34	223.0±42.37	575.7±32.41
Serratus anterior	6	50	352.3±18.23	405.2±35.38	162.2±44.70	567.3±31.73
Deltoid	5	29	335.2± 8.35	379.8±37.25	170.8±41.84	550.6±16.80
Latissimus dorsi	6	49	349.5±18.04	406.3±19.48	160.8±32.37	567.1±43.21
Tensor Fasciae latae	5	37	348.8±18.57	398.6±28.13	161.6±22.09	560.2±17.75
Gluteus maximus	3	35	354.5	389.5	181.4	570.9
Gluteus medius	2	44	340.5	370.0	166.5	536.5
Vastus medialis	2	36	340.5	422.9	140.5	563.4
Rectus femoris	2	23	334.8	420.0	122.2	542.2
Rhomoid major	2	41	346.4	361.2	221.0	582.2
Adductor longus	1	64	380.8	398.6	182.7	581.3
Adductor brevis	1	64	380.8	463.0	132.2	595.2
Iliacus	1	31	338.6	428.1	135.1	563.2
Ext. obliq. abd.	1	68	336.7	360.3	165.9	526.2
Tibialis posterior	1	29	348.4	406.7	144.8	551.5
Serratus post. inf.	1	60	356.6	445.2	128.3	573.5
Pyramidalis abd.	1	48	358.7	424.3	181.2	605.5
Sartorius	2	14	344.7	405.3	144.3	549.6
TOTAL	131	44	351.8±16.22	405.0±35.30	163.3±41.76	567.3±30.28

TABLE 8 (A)

RESULTS OF ANALYSIS OF VARIANCE FOR 10 GROUPS OF MUSCLES

Groups of muscle	No. of Samples	Water (FFWW)	K (FFWW)	Na (FFWW)	K+Na (FFWW)
10	106	F = 1.47	F = 5.26	F = 3.62	F = 0.53
		P = N.S.	P<0.01	P<0.01	P = N.S.

TABLE 8 (B)

RESULTS OF ANALYSIS OF VARIANCE FOR 10 GROUPS OF MUSCLES

Groups of muscles	No. of Samples	Water (FFDW)	K (FFDW)	Na (FFDW)	K+Na (FFDW)
10	106	F = 1.80	F = 8.93	F = 2.81	F = 0.94
		P = N.S.	P<0.01	P<0.01	P = N.S.

F: Variance ratio.

N.S.: not significant.

10 groups of muscles: pectoralis major, pectoralis minor, rectus abdominis, vastus lateralis, vastus intermedius, erector spinae, intercostal, serratus anterior, deltoid and latissimus dorsi.

TABLE 9 (A) DATA OF COMPOSITION IN MUSCLES TAKEN FROM DIFFERENT SITES IN EACH SUBJECT

Case No.	Sex	Age	*Δ Intercostal muscle				*○ Serratus anterior				Δ○ Latissimus dorsi			
			Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Water ml/100g FFWW	K mEq/kg FFDW	Na mEq/kg FFWW	K+Na mEq/kg FFWW
1	F	54	78.07	77,78	51.95	129.73	77.54	90.59	42.30	132.89	--	--	--	--
2	M	61	77.64	71.64	53.28	124.92	--	--	--	--	77.46	84.29	33.46	117.75
3	F	56	77.38	84.14	39.62	123.76	77.61	93.64	35.49	127.13	77.15	93.46	31.42	124.88
4	M	54	78.71	82.35	45.93	128.28	78.48	93.92	34.58	128.50	79.01	85.90	45.69	131.59
5	F	29	76.62	82.66	42.08	124.74	76.32	96.12	24.08	120.20	76.47	94.23	32.58	126.81

** Comparison of paired observations analysis by t-test for Intercostal m. & Serratus anterior

	Water	K	Na	K+Na
t	1.30	13.58	4.53	0.30
p	N.S.	P<0.001	P<0.05	N.S.

ΔΔ Comparison of paired observations analysis by t-test for Intercostal m. and Latissimus dorsi.

	Water	K	Na	K+Na
t	0.53	4.57	2.34	0.07
p	N.S.	P<0.05	N.S.	N.S.

○○ Comparison of paired observations analysis by t-test for Serratus anterior and Latissimus dorsi.

	Water	K	Na	K+Na
t	0.53	1.41	0.79	0.96
p	N.S.	N.S.	N.S.	N.S.

TABLE 9 (B) DATA OF COMPOSITION IN MUSCLES TAKEN FROM DIFFERENT SITES IN EACH SUBJECT

Case No.	Sex	Age	*Δ Intercostal muscle				*o Serratus anterior				Δo Latissimus dorsi			
			Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW
1	F	54	356	355	237	592	345	403	188	591	--	--	--	--
2	M	61	347	320	238	559	--	--	--	--	344	374	148	522
3	F	56	342	372	175	547	347	418	150	568	338	409	137	546
4	M	54	370	387	216	603	365	436	161	597	376	409	218	627
5	F	29	328	354	180	534	322	406	102	508	325	400	138	538

** Comparison of paired observations analysis by t-test for Intercostal m. and Serratus anterior.

	Water	K	Na	K+Na
t	1.27	39.0	4.75	0.31
p	N.S.	P<0.001	P<0.05	N.S.

ΔΔ Comparison of paired observations analysis by t-test for Intercostal m. and Latissimus dorsi.

	Water	K	Na	K+Na
t	0.43	5.79	2.23	0.20
p	N.S.	P<0.05	N.S.	N.S.

oo Comparison of paired observation analysis by t-test for Serratus anterior and Latissimus dorsi.

	Water	K	Na	K+Na
t	0.29	1.19	1.31	0.73
p	N.S.	N.S.	N.S.	N.S.

In spite of variation in potassium and sodium content in the muscle, the sum of potassium plus sodium remains constant. Muscle sodium was plotted against muscle potassium with FFWW as the basis of reference in the Figure 1 (A) and FFDW in the Figure 1 (B). They show that there is a negative correlation between sodium and potassium in these 131 samples. When reported as mEq per kg FFWW, the linear regression equation is $Y = 125.64 - X$, $r = -0.88$, $P<0.001$. (Y = muscle sodium mEq/kg FFWW, X = muscle potassium mEq/kg FFWW). When expressed with mEq per kg FFDW the linear regression equation is $Y = 518.66 - 0.88X$, $r = -0.74$, $P<0.001$ (Y = muscle sodium mEq/kg FFDW, X = muscle potassium mEq/kg FFDW).

A positive correlation between the muscle content of water and the sum of sodium plus potassium is also demonstrated at terms of FFDW as shown in Figure 2 (B). The linear regression equation is $Y = 1.41 X + 71.25$, $r = 0.76$, $P<0.001$. (X = water ml/100g FFDW, Y = the sum of sodium plus potassium in the muscle mEq/kg FFDW).

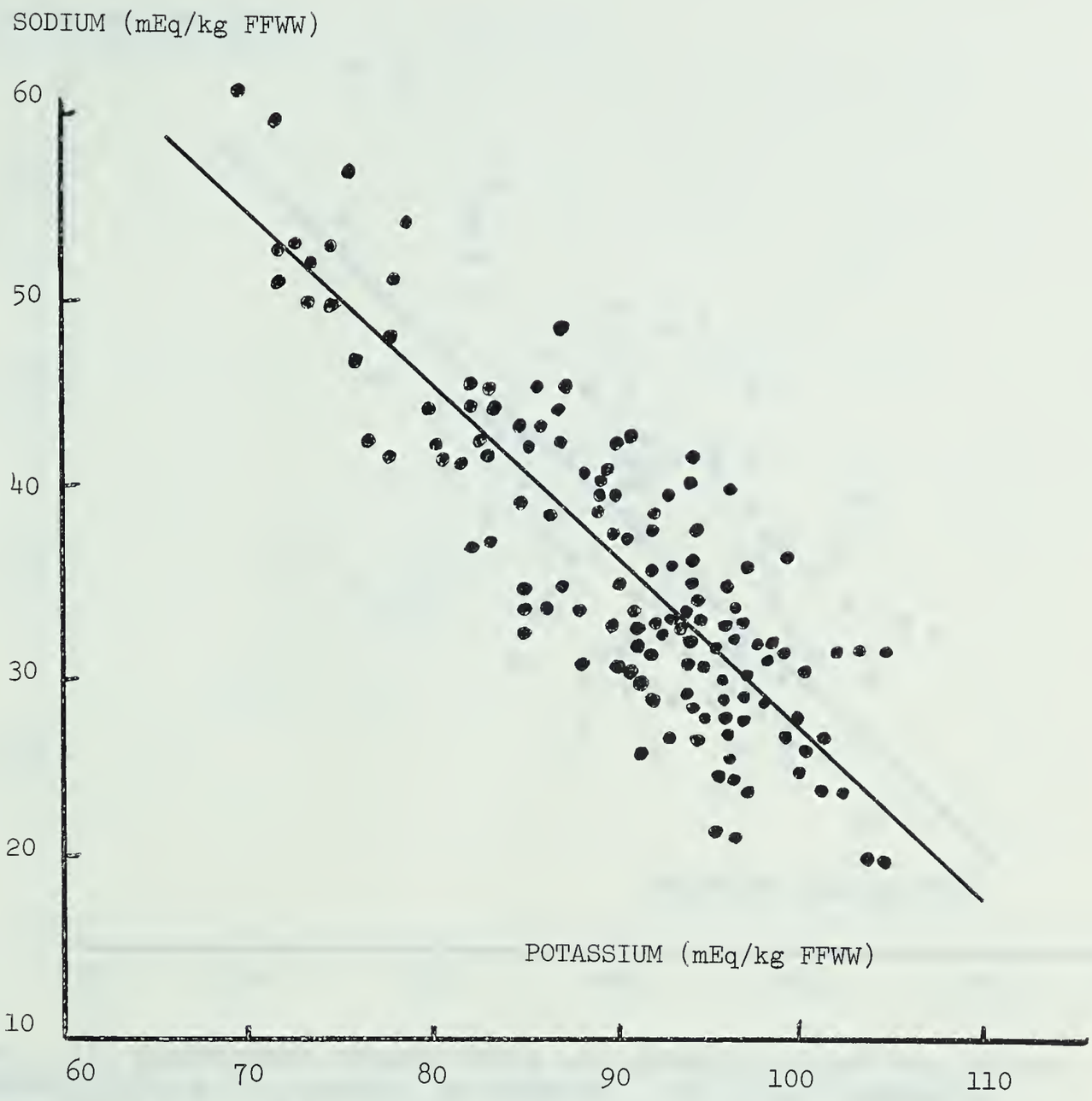
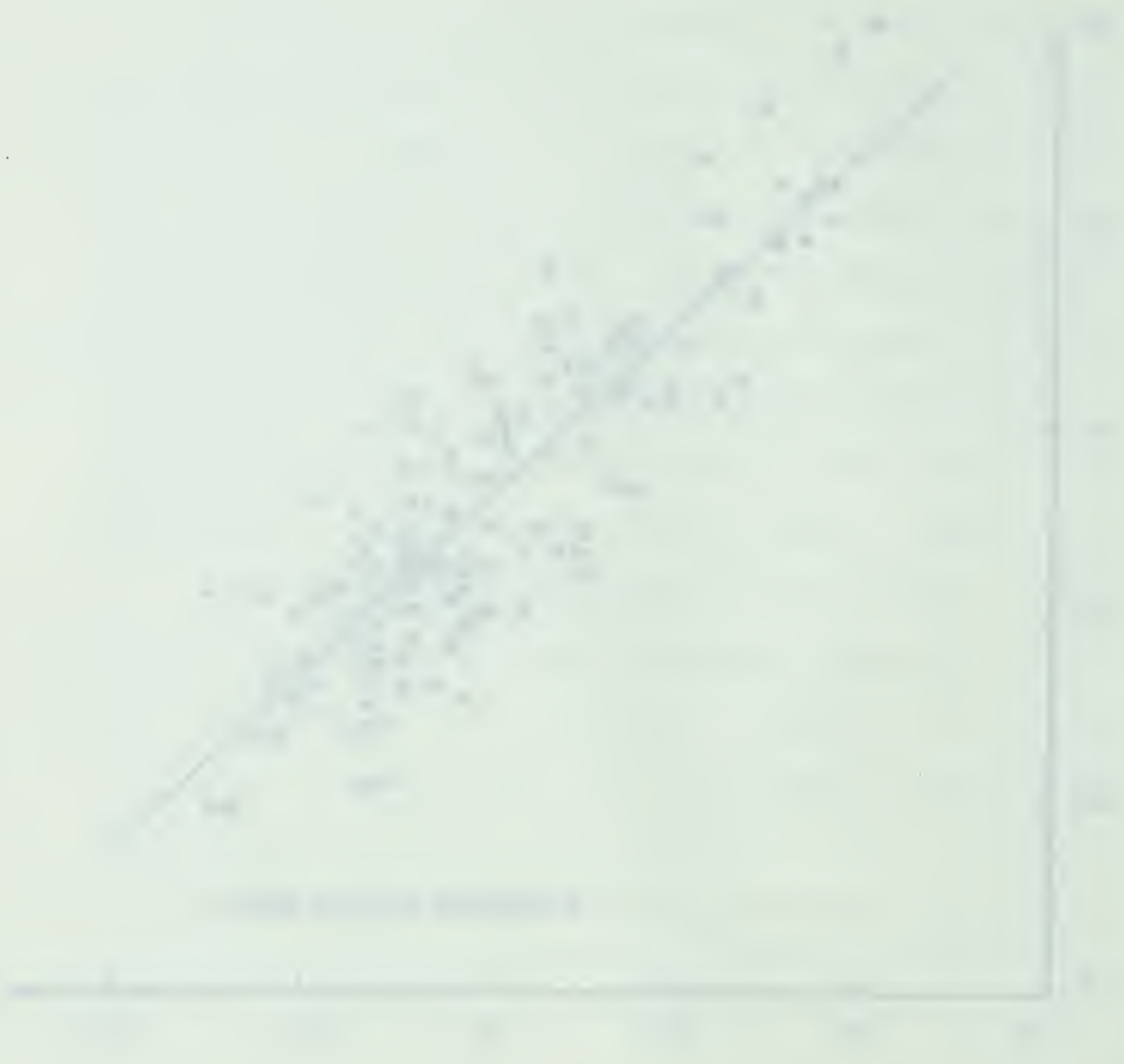


FIG. 1(A) RELATIONSHIP BETWEEN SODIUM AND POTASSIUM CONTENT FOR ADULT'S SKELETAL MUSCLE (131 SAMPLES, 89 SUBJECTS). THE LINEAR REGRESSION EQUATION IS Na = 125.64 - K. r = -0.88 P<0.001



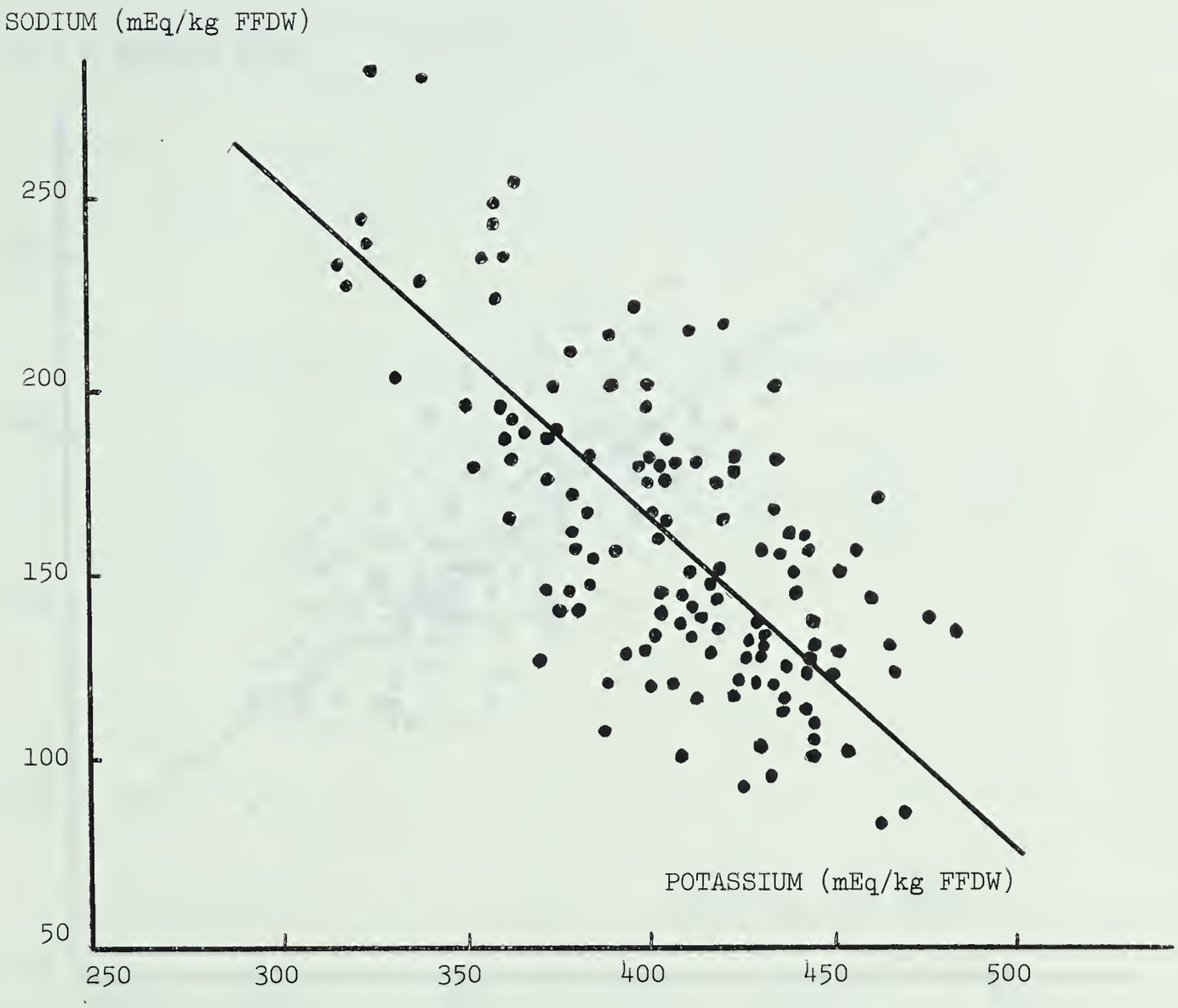


FIG. 1(B) RELATIONSHIP BETWEEN SODIUM AND POTASSIUM CONTENT FOR ADULT'S SKELETAL MUSCLE (131 SAMPLES, 89 SUBJECTS). THE LINEAR REGRESSION EQUATION IS Na = 518.66 - 0.88K. $r = -0.74$, $P<0.001$.

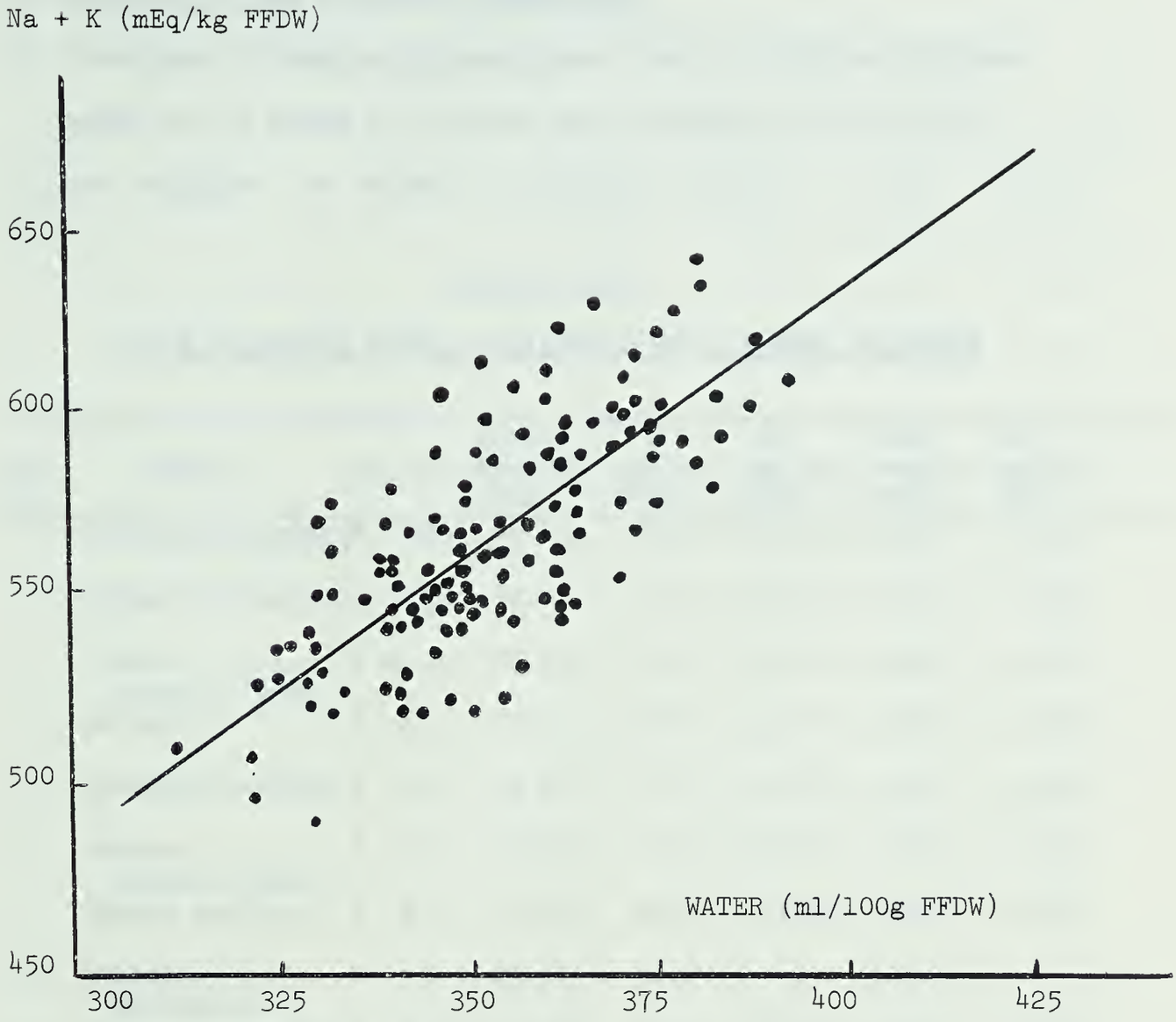


FIG. 2(B) RELATIONSHIP BETWEEN MUSCLE CONTENT OF WATER AND THE SUM OF SODIUM PLUS POTASSIUM FOR ADULT'S SKELETAL MUSCLE (131 SAMPLES, 89 SUBJECTS). THE LINEAR REGRESSION EQUATION IS (Na+K) = 1.41 WATER + 71.25. $r=0.76$, $P<0.001$.

(B) Relation of Age to Muscle Composition

(1) Young Age: 17 muscle biopsies taken from 17 children with age range from 7 weeks to 13 years were analyzed and the results are tabulated in Tables 10 (A) and 10 (B).

TABLE 10 (A)

DATA OF MUSCLE BIOPSY OBTAINED FROM 17 NORMAL CHILDREN

Case No.	Muscle	Sex	Age	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Fat g/100g WW
1	Pectoralis major	M	7wks	81.75	74.01	55.51	129.60	0.02
2	Pectoralis major	M	5 mo	78.65	75.73	48 50	124.23	0.00
3	Tensor fasciae latae	M	21 mo	79.91	63.73	60.83	124.56	0.04
4	Deltoid	M	2 yr	79.43	73.35	52.15	125.50	0.00
5	Serratus anterior	M	2 yr	78.24	77.34	47.81	125.15	0.00
6	Tensor fasciae latae	F	4 yr	80.35	75.18	59.31	134.49	0.01
7	Vastus medialis	M	4 yr	76.72	69.86	57.68	127.54	0.05
8	Tibialis posterior	F	4 yr	79.37	81.29	46.43	127.72	--
9	Pectoralis major	M	5 yr	77.28	82.04	45.07	127.11	0.01
10	Vastus lateralis	F	5 yr	78.65	57.36	73.42	130.78	0.01
11	Sartorius	M	5 yr	79.09	83.85	50.91	134.76	1.14
12	Erector spinae	F	9 yr	79.48	74.72	53.41	128.13	0.00
13	Erector spinae	F	11 yr	77.86	76.79	49.43	126.22	0.06
14	Erector spinae	F	13 yr	78.68	80.26	48.79	129.05	0.04
15	Vastus lateralis	F	13 yr	78.71	78.50	44.45	122.95	2.67
16	Vastus lateralis	M	13 yr	78.21	83.65	34.66	118.31	2.18
17	Gluteus medius	M	13 yr	79.43	77.29	42.01	119.30	4.88
	Mean			78.93	75.59	51.20	126.79	0.69 (N=16)
N=17	S. D.			1.18	6.89	8.70	4.43	1.40

TABLE 10 (B)

DATA OF MUSCLE BIOPSY OBTAINED FROM 17 NORMAL CHILDREN

Case No.	Muscle	Sex	Age	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW
1	Pectoralis major	M	7 wk	447.95	405.53	304.16	707.69
2	Pectoralis major	M	5 mo	368.38	354.71	227.17	581.88
3	Tensor fasciae latae	M	21 mo	397.76	317.22	302.79	620.01
4	Deltoid	M	2 yr	386.14	356.59	253.52	610.11
5	Serratus anterior	M	2 yr	359.56	355.42	219.72	575.14
6	Tensor fasciae latae	F	4 yr	408.91	382.59	301.83	684.42
7	Vastus medialis	M	4 yr	329.55	300.09	247.77	547.86
8	Tibialis posterior	F	4 yr	384.73	394.04	225.06	619.10
9	Pectoralis major	M	5 yr	340.14	361.09	198.37	559.46
10	Vastus lateralis	F	5 yr	368.38	268.67	343.89	612.56
11	Sartorius	M	5 yr	378.24	401.00	243.47	644.47
12	Erector spinae	F	9 yr	387.33	364.13	260.28	624.41
13	Erector spinae	F	11 yr	351.67	346.84	223.26	570.10
14	Erector spinae	F	13 yr	369.04	376.45	228.85	605.30
15	Vastus lateralis	F	13 yr	369.70	368.72	208.78	577.50
16	Vastus lateralis	M	13 yr	358.93	383.89	159.06	542.95
17	Gluteus medius	M	13 yr	386.14	375.74	204.23	579.97
N = 17	Mean			375.94	359.59	244.18	603.76
	S.D.			27.22	35.83	46.71	45.39

The muscle composition of children is quite different from that of adults. All data of this comparison are tabulated in Tables 11 (A) and 11 (B). The sample size of pectoralis major, vastus lateralis and erector spinae in the chidren are larger as compared to that of other. These 3 groups of muscle which the sample size ratios of children to adults are 3:11, 3:11 and 3:10 are shown in Table 11 (A) and (B). Therefore a comparison of sample means by t-test may be applied to study these 9 children and 32 adults muscles. The results of analysis show that when expressed in FFWW or FFDW the water and sodium content in children's muscle are significantly higher than those in adults, and the potassium content is significantly lower than that in adults' muscle. When the values are expressed with FFWW, no significant difference of the sum of potassium plus sodium in the muscle between children and adults is noted, and the negative correlation shown in Figure 3 (A) between sodium and potassium is well demonstrated; however, when the results are expressed with FFDW, a significant increase in the sum of potassium plus sodium in the muscle of children as compared with that of adults is revealed, and the negative correlation as shown in Figure 3 (B) between sodium and potassium is no longer present. The positive correlation between muscle content of water and the sum of sodium plus potassium is established as shown in Figure 4 (B) when expressed with FFDW.

There are 6 muscle biopsies (3 erector spinae, 2 vastus lateralis, 1 gluteus medius) taken from children ranging in age from 9 to 13 years. If one compares the mean value of these 6 muscle biopsies with that of the same muscle groups in adults by t-test, a significant difference is found. It is still evident that sodium and water content are higher and potassium content is lower in the muscle of prepubertal

TABLE 11 (A)

COMPARISON OF MUSCLE COMPOSITION BETWEEN ADULTS AND CHILDREN

No. of Cases	Ave Age	Site	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Fat g /100g WW
11	44 yr	Pectoralis major	77.81	94.75	29.95	124.70	0.87
3	22 mo	" "	79.27	77.26	49.69	126.95	0.01
11	33 yr	Vastus lateralis	77.92	90.72	33.73	124.45	1.93
3	10 yr	" "	78.52	73.17	50.84	124.01	1.62
10	41 yr	Erector spinae	77.80	85.63	37.60	123.23	4.21
3	11 yr	" "	78.67	77.26	50.54	127.80	0.03
2	36 yr	Vastis medialis	77.26	96.01	31.93	127.94	1.03
1	4 yr	" "	76.72	69.86	57.68	127.54	0.05
4	28 yr	Tensor fasciae latae	77.33	89.65	36.87	126.52	0.64
2	2 yr	" " "	80.13	69.45	60.07	129.52	0.04
2	15 yr	Sartorius	77.50	91.20	32.30	123.50	0.03
1	5 yr	"	79.09	83.85	50.91	134.76	1.14
6	50 yr	Serratus anterior	77.86	89.70	36.05	125.75	2.45
1	2 yr	" "	78.24	77.34	47.81	125.15	0.00
2	45 yr	Glateus medius	77.27	83.96	37.82	121.78	4.91
1	13 yr	" "	79.43	77.29	42.01	119.30	4.88
5	29 yr	Deltoid	77.02	87.03	39.15	126.18	1.19
1	2 yr	"	79.43	73.35	52.15	125.50	0.00
1	29 yr	Tibialis posterior	77.71	90.69	32.29	122.97	2.27
1	1 yr	" "	79.37	81.29	46.43	127.72	--

Age Group	N	Mean ± S.D.				
Children	9	78.80±1.21	75.90±7.69	50.36±10.50	126.26±3.90	0.55±1.07
Adults	32	77.81±0.85	90.98±7.65	33.47±7.51	124.45±3.80	2.16±2.46
Comparison of 2 sample means by t-test for 3 larger groups of muscle (P Ma, VL & ES)		t=2.78 P<0.01	t=5.22 P<0.001	t=5.45 P<0.001	t=1.25 P=N.S.	t=1.90 P=N.S.

*No muscle taken from patient over 60 years of age in this table.
*If possible, the mean muscle value will be calculated as follows: mean muscle value = (mean muscle value of male + mean muscle value of female)÷ 2.
*P Ma: Pectoralis major. VL: Vastus Lateralis. ES: Erector Spinae.

TABLE 11 (B)

COMPARISON OF MUSCLE COMPOSITION BETWEEN ADULTS & CHILDREN

No. of Cases	Ave Age	Site	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW
11	44 yr	Pectoralis major	350.65	426.99	134.97	561.96
3	22 mo	" "	382.39	372.70	239.70	612.40
11	33 yr	Vastus lateralis	352.90	410.87	152.76	563.63
3	10 yr	" "	365.55	340.64	236.68	577.32
10	41 yr	Erector spinae	350.45	385.72	169.37	555.09
3	11 yr	" "	368.82	362.21	236.94	599.15
2	36 yr	Vastus medialis	339.75	422.21	140.41	562.62
1	4 yr	" "	329.55	300.08	247.77	547.85
4	28 yr	Tensor " fasciae latae	341.25	390.50	163.00	553.50
2	2 yr	" " "	403.27	349.52	302.32	651.84
2	15 yr	Sartorius	344.70	405.30	144.30	549.60
1	5 yr	"	378.24	401.00	243.47	644.47
6	50 yr	Serratus anterior	351.67	405.14	162.83	567.97
1	2 yr	" "	359.56	355.42	219.72	575.14
2	45 yr	Gluteus medius	339.95	369.38	166.39	535.77
1	13 yr	" "	386.14	375.74	204.23	579.97
5	29 yr	Deltoid	335.16	378.72	170.37	549.09
1	2 yr	"	386.14	356.59	253.52	610.11
1	29 yr	Tibialis posterior	348.63	406.81	144.86	551.67
1	1 yr	" "	384.73	394.04	225.06	619.10

Age Group	N	Mean ± S.D.			
Children	9	373.44±30.86	359.00±37.93	239.22±56.08	598.22±49.34
Adults	32	351.31±16.92	410.03±30.89	151.31±37.78	561.34±28.26
Comparison of 2 sample means by t-test for 3 larger groups of muscle (P Ma, VL & ES)		t=2.85	t=4.71	t=5.52	t=2.9
		P<0.01	P<0.001	P<0.001	P<0.01

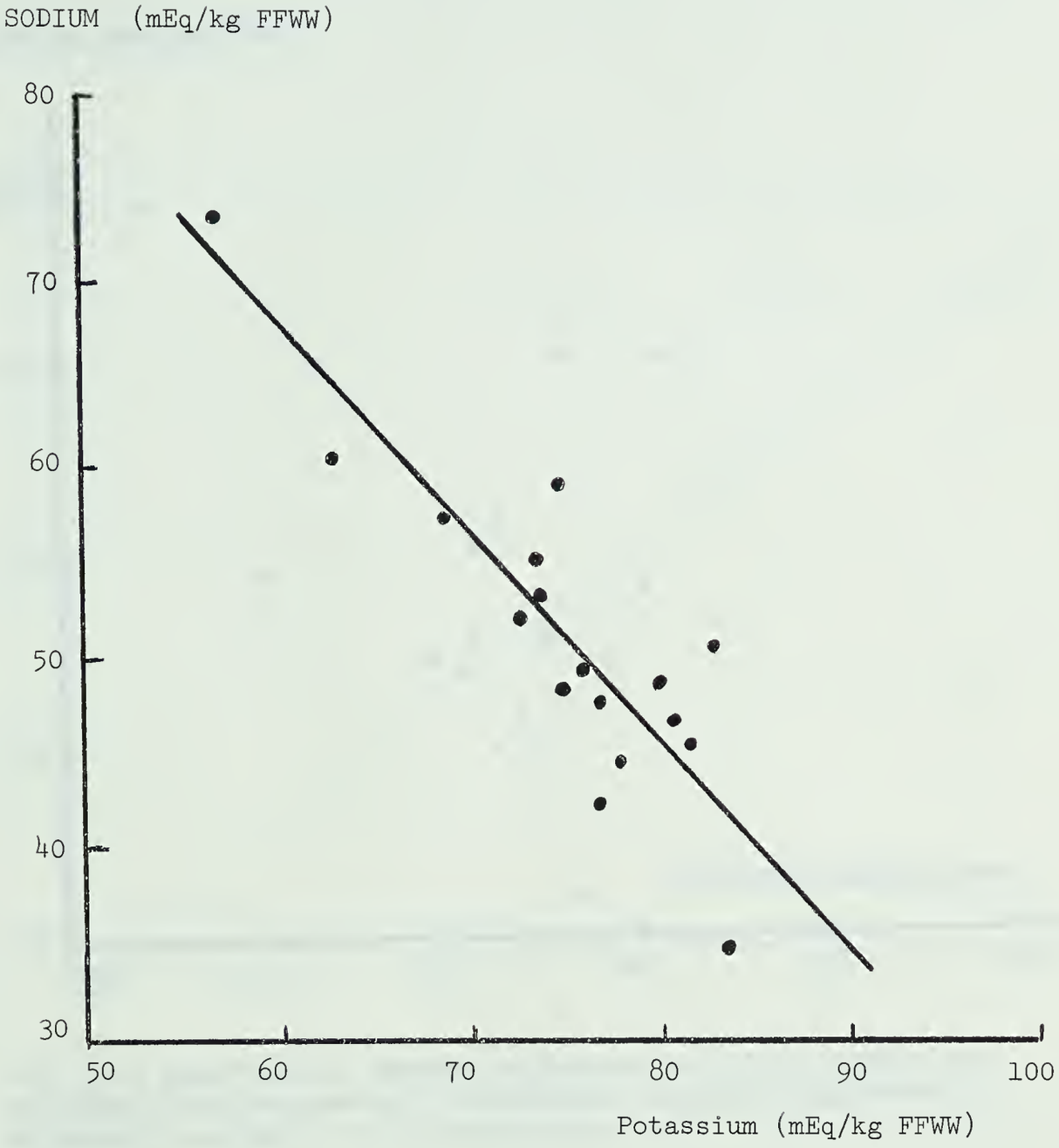


FIG. 3(A) RELATIONSHIP BETWEEN SODIUM AND POTASSIUM CONTENT FOR CHILDREN'S SKELETAL MUSCLE. THE LINEAR REGRESSION EQUATION IS Na = 133.59 - 1.09K. r = -0.86 P<0.001.

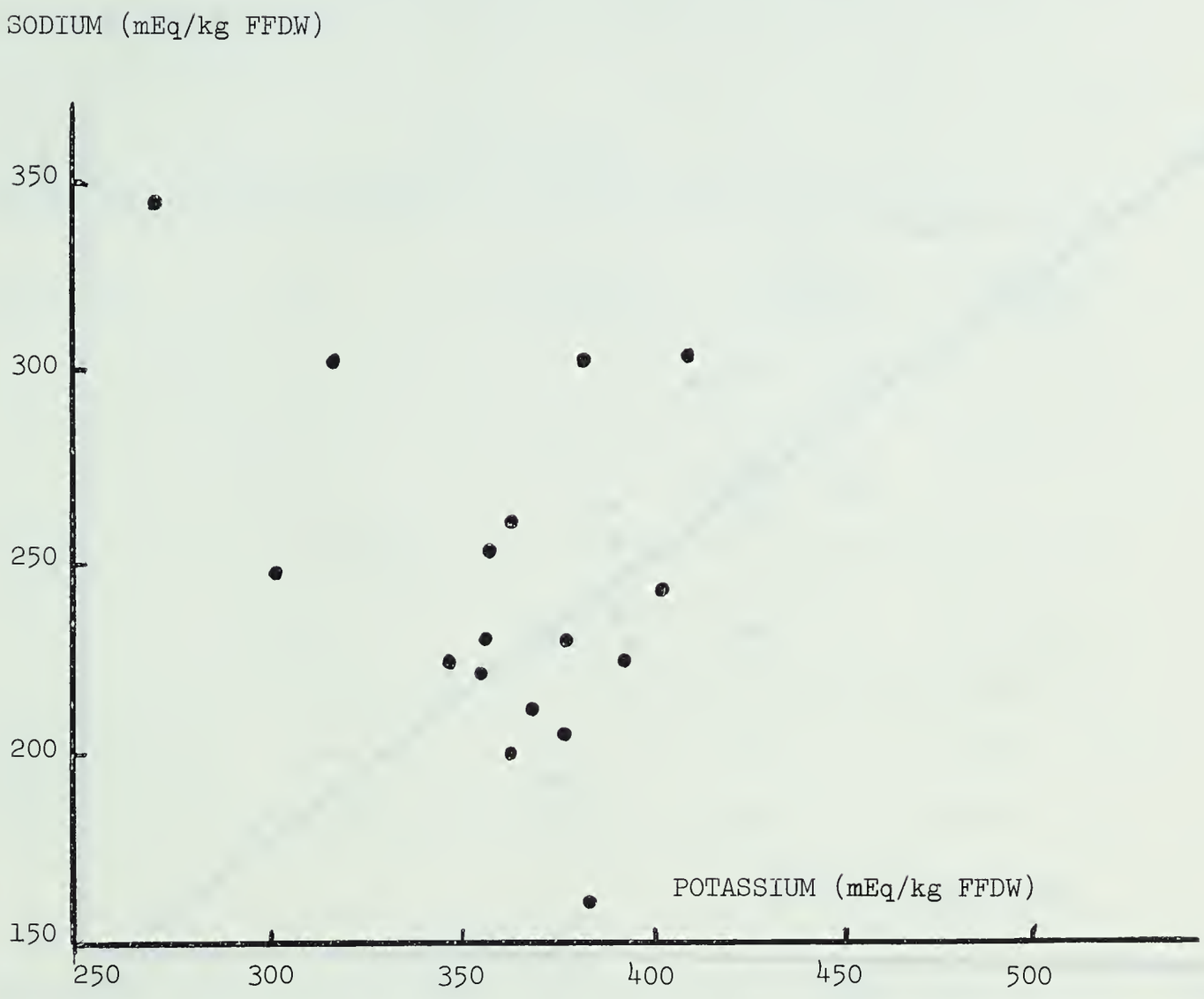


FIG. 3(B) RELATIONSHIP BETWEEN SODIUM AND POTASSIUM CONTENT FOR CHILDREN'S SKELETAL MUSCLE. THE LINEAR REGRESSION EQUATION IS Na = 440.9 - 0.547K. r = -0.42, P>0.05.

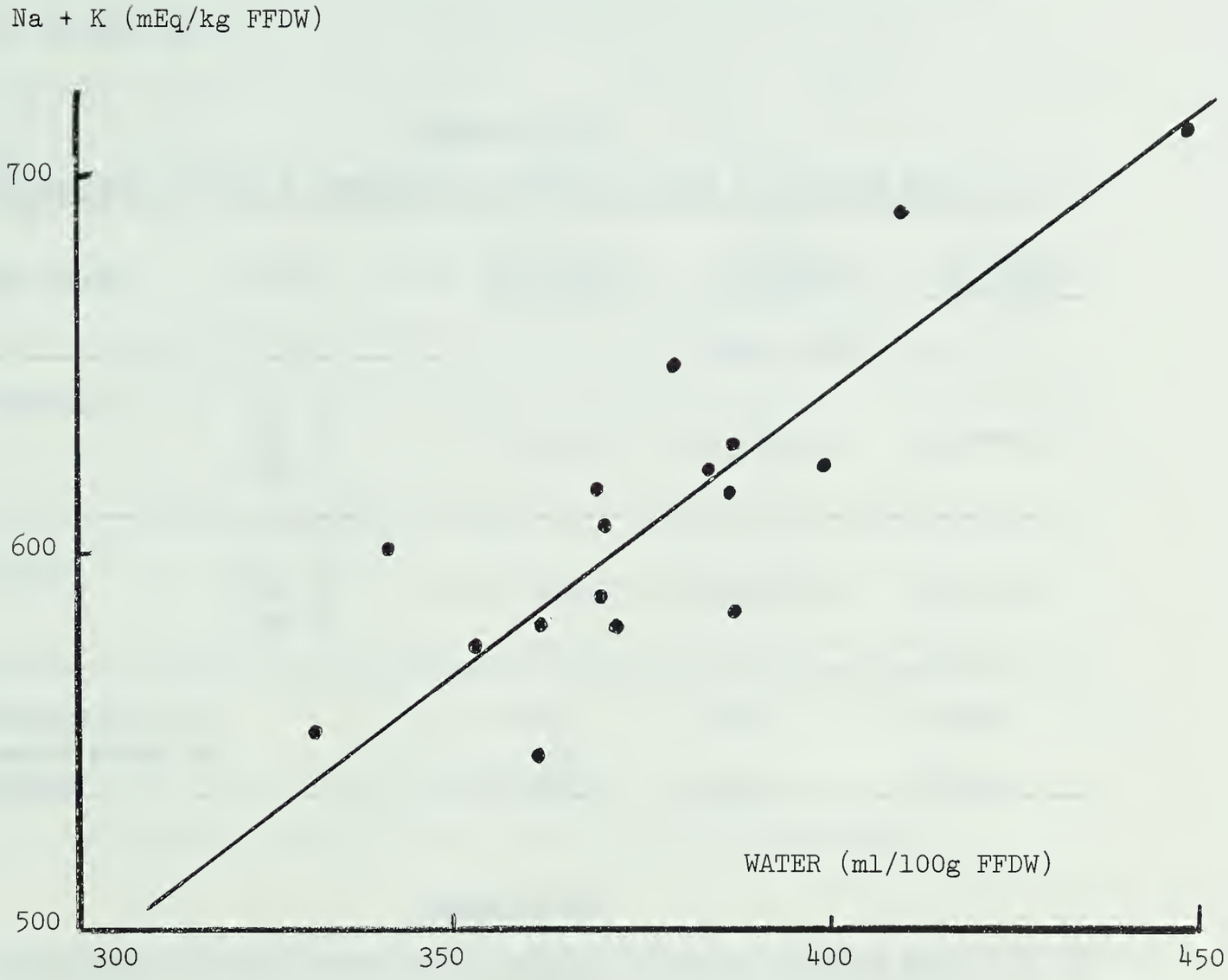


FIG. 4(B) RELATIONSHIP BETWEEN MUSCLE CONTENT OF WATER AND THE SUM OF SODIUM PLUS POTASSIUM FOR CHILDREN'S SKELETAL MUSCLE. THE LINEAR REGRESSION EQUATION IS (Na+K) = 1.49 WATER + 43.7.
r=0.89, P<0.001

children than in those of adults. The results are shown in Tables 12 (A) and (B).

TABLE 12 (A)

COMPARISON OF MUSCLE COMPOSITION BETWEEN ADULTS & CHILDREN AGES 9 TO 13

Age Group	Site	N	Water ml/ 100g FFWW	K mEq/ kg FFWW	Na mEq/ kg FFWW
			Mean ± S.D.		
Children	ES 3 VL 2 GMe 1	6	78.73±0.64	78.53±3.11	45.46±6.63
Adults	ES 10 VL 11 GMe 2	23	77.76±0.97	88.56±7.74	35.54±7.31
Comparison of 2 sample means by t-test		t	2.25	3.01	2.96
		P	P<0.05	P<0.01	P<0.01

TABLE 12 (B)

COMPARISON OF MUSCLE COMPOSITION BETWEEN ADULTS & CHILDREN AGES 9 TO 13

Age Group	Site	N	Water ml/ 100g FFDW	K mEq/ kg FFDW	Na mEq/ kg FFDW
			Mean ± S.D.		
Children	ES 3 VL 2 GMe 1	6	370.50±14.06	369.33±12.89	214.00±33.37
Adults	ES 10 VL 11 GMe 2	23	350.52±19.72	398.26±30.31	160.30±37.80
Comparison of 2 samples means by t-test		t	2.27	2.22	3.10
		P	P<0.05	P<0.05	P<0.01

(2) Old Age In a total of 89 adult biopsies, only a few were obtained from patients over 60 years of age. Pectoralis major is the largest sample size among all muscle groups. A total of 18 samples of pectoralis major were divided into 2 groups, averaging 44 and 72 years of age and tabulated in Tables 13 (A) and 13 (E). When the results are expressed with FFWW, it is found that the water content of muscle in the older age group is significantly different ($t = 2.58$, $P<0.05$) from that in the young age group. However, when the values are reported as FFDW, it is found that not only is the water content significantly increased ($t = 2.52$, $P<0.05$) but also the sodium content ($t = 2.18$, $P<0.05$) and the sum of potassium plus sodium ($t = 2.65$, $P<0.05$) in the muscle of the old age group as compared to the young age group. A significant difference at the 1% level ($t = 3.3$) is an increase in neutral fat in the old age groups when expressed with g per 100g wet weight.

(C) Difference of Muscle Composition between Female and Male

7 groups of muscle in which the difference of average age between female and male within each group is not more than 5 years have been chosen for analysis of the difference of muscle composition between the sexes. The data are shown in Tables 14 (A) and 14 (B). The sample sizes in these groups of muscle are small and unequal. Therefore, Wilcoxon's Signed Rank Test (29,32) is applied. These seven groups of muscle are analyzed by comparing seven mean values for females with those of males. It is shown that the amount of water in muscle per kg FFWW or per kg FFDW is relatively greater in female than in male. When the value of the sum of sodium plus potassium is expressed as FFDW, a sex difference is demonstrable, the value being greater for female than male. No significant difference of sodium or potassium content in the muscles between sexes is found.

TABLE 13 (A)

MUSCLE VALUES IN 18 NORMAL SUBJECTS
RESULTS OBTAINED FROM THE SPECIMENS OF PECTORALIS MAJOR

Case No.	Age	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Fat g /100g WW
5	73	78.08	91.53	31.61	123.14	2.89
8	60	79.14	90.61	42.17	132.78	3.00
9	86	78.54	98.41	36.84	135.25	2.98
11	79	77.59	94.37	41.38	135.75	8.19
13	74	78.88	92.79	32.25	125.04	2.94
16	70	78.25	86.11	48.96	135.07	0.82
18	63	78.29	95.77	24.38	120.15	1.71
1	45	77.61	99.35	24.01	123.36	1.01
2	40	77.27	90.11	29.97	120.08	0.19
3	53	78.04	91.22	28.84	120.06	0.45
4	36	77.59	95.14	21.09	116.23	1.31
6	47	78.47	81.60	45.50	127.10	1.17
7	45	77.51	99.31	29.46	128.77	1.04
10	56	77.55	99.62	25.88	125.50	0.79
12	44	77.79	95.33	31.48	126.81	0.69
14	55	78.61	101.70	30.26	131.96	1.35
15	18	77.30	93.86	35.30	129.16	1.00
17	48	78.20	95.06	27.69	122.75	0.60

Age group	N	Water Mean±S.D.	K Mean±S.D.	Na Mean±S.D.	K+Na Mean±S.D.	Fat Mean±S.D.
60 or over	7	78.40±0.50	92.80±3.96	36.80±8.16	129.57±6.60	3.22±2.35
Under 60	11	77.81±0.45	94.75±6.41	29.95±6.40	124.71±4.69	0.87±0.35
t-test	t	2.58	0.79	1.99	1.83	3.3
	p	P<0.05	N.S.	N.S.	N.S.	P<0.01

TABLE 13 (B)

MUSCLE VALUES IN 18 NORMAL FEMALE SUBJECTS
RESULTS OBTAINED FROM SPECIMENS OF PECTORALIS MAJOR

Case No	Age	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW
5	73	356.20	417.56	144.21	561.77
8	60	379.39	434.37	202.16	636.53
9	86	365.98	458.57	171.67	630.24
11	79	346.23	421.11	184.65	605.76
13	74	373.48	439.35	152.70	592.05
16	70	359.77	395.91	225.10	621.01
18	63	360.62	441.13	112.30	553.43
1	45	346.63	443.72	107.24	550.96
2	40	339.95	396.44	131.85	528.29
3	53	355.37	415.39	131.33	546.72
4	36	346.23	424.54	94.11	518.65
6	47	364.47	379.01	211.33	590.34
7	45	344.64	441.57	130.99	572.56
10	56	345.43	443.74	115.28	559.02
12	44	350.24	429.22	141.74	570.96
14	55	367.51	475.46	141.46	616.92
15	18	340.53	413.48	155.51	568.99
17	46	358.72	436.06	127.02	563.07

Age Group	N	Water Mean±S.D.	K Mean±S.D.	Na Mean±S.D.	K+Na Mean±S.D.
60 or over	7	363.10±20.17	429.71±20.17	170.40±37.82	600.11±32.69
Under 60	11	350.88± 9.37	427.15±26.02	135.26±30.44	562.41±27.28
t-test	t	2.52	0.22	2.18	2.65
	p	P<0.05	N.S.	P<0.05	P<0.05

TABLE 14 (A)

COMPARISON OF 7 GROUPS OF MUSCLE VALUES BETWEEN FEMALE AND MALE

No. of Cases	Sex	Ave Age	Sites	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Fat g /100g WW
7	F	43	Rectus abdominis	77.6	88.5	38.3	126.8	2.64
3	M	46	" "	76.7	90.5	31.2	121.1	1.29
2	F	27	Vastus lateralis	78.6	87.5	39.2	126.7	1.38
4	M	24	" "	77.5	92.5	31.8	124.3	2.57
3	F	49	Latissimus dorsi	78.5	90.3	38.8	129.0	3.43
3	M	48	" "	77.0	90.7	32.4	123.1	1.22
2	F	20	Vastus intermedius	78.8	84.4	37.8	122.2	0.26
2	M	25	" "	77.5	88.9	38.9	127.8	0.97
2	F	39	Erector spinae	78.3	82.4	38.3	120.7	3.58
6	M	36	" "	77.1	87.8	34.1	121.9	2.42
3	F	56	Intercostal	78.5	76.5	53.1	129.6	3.51
2	M	58	"	77.6	77.9	46.5	124.3	4.80
3	F	54	Serratus anterior	78.3	91.8	35.5	127.2	3.24
2	M	54	" "	78.0	83.4	42.9	126.3	2.00
Wilcoxon's Signed-			T	0	7	7.5	7	10
Rank Test (N=7)			P	P<0.02	N.S.	N.S.	N.S.	N.S.

TABLE 14 (B)

COMPARISON OF 7 GROUPS OF MUSCLE VALUES BETWEEN FEMALE AND MALE

No of Cases	Sex	Ave Age	Sites	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW
7	F	43	Rectus abdominis	345.83	394.65	170.58	565.23
3	M	46	" "	328.82	388.12	133.83	521.95
2	F	27	Vastus lateralis	366.42	407.98	182.98	590.96
4	M	24	" "	343.85	410.56	140.97	551.53
3	F	49	Latissimus dorsi	364.25	419.03	180.04	599.07
3	M	48	" "	335.35	394.69	141.45	536.14
2	F	20	Vastus intermedius	371.25	397.69	178.23	575.92
2	M	25	" "	343.66	394.63	172.63	567.26
2	F	39	Erector spinae	360.41	379.33	176.38	555.71
6	M	36	" "	337.25	383.82	149.19	533.01
3	F	56	Intercostal	364.68	355.58	246.70	602.28
2	M	58	"	346.83	348.03	207.55	555.59
3	F	54	Serratus anterior	360.19	422.32	163.14	585.46
2	M	54	" "	354.19	379.13	195.23	574.36
Wilcoxon's Signed - Rank Test (N=7)			T	0	4	3	0
			P	P<0.02	N.S.	N.S.	P<0.02

(D) Abnormal Muscle Water, Sodium and Potassium Content due to Diseases

8 muscle biopsies were obtained from patients with complications of muscle wsting, cardiac or renal disease. The results are presented in Tables 15 (A) and 15 (B). Most of these cases demonstrate an increase in water and sodium content in the muscles with a decrease in muscle potassium concentration as compared to normal adult. An increase in the sum of potassium plus sodium is also observed in all cases when the values are expressed as FFDW.

IV. DISCUSSION

(A) Sample Sizes and Sites

In the study reported in this paper, the sample sizes were comparatively similar, averaging 0.14 to 0.16 g in weight. 124 duplicate samples have been analyzed. The larger slices of duplicate samples were grouped and the smaller samples compared in a second group. Using paired observations analysis by t-test (29). No significant difference of potassium, sodium or water content between the muscles of these 2 groups was determined. The results of the t-test are shown in the Tables 16 (A) and 16 (B).

During the course of this study, Johnston in 1965 (36) reported his findings on the muscle electrolyte composition employing biopsies ranging in weight from 20 to 1500 mg of dog muscle and using a single muscle origin. He reported that total potassium in mEq/100 g fat free, blood free, dry solid (FFBFDS) bore a direct hyperbolic relationship to the weight of the biopsy sample and that total sodium content bore an inverse hyperbolic relationship to the weight of dry solid (in mEq/100g FFBFDS). Flear et al. (23) compared the values of sodium and potassium in rat

TABLE 15 (A)

DATA OF MUSCLE BIOPSY FROM KIDNEY, HEART OR MUSCLE ATROPHIC PATIENTS

Case No.	Muscle	Sex	Age	Disease and Clinical Signs	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	K+Na mEq/kg FFWW	Fat g /100g FFWW
1	Tensor fasciae latae	M	43	Spinal cord injury in 1958. Muscle atrophy, quadriplegia. Fracture of femur	79.33	65.24	62.72	127.95	3.16
2	Gluteus medius	M	57	Fracture of femur in 1963. Disuse muscle atrophy, marked wasting of the thigh musculature	80.55	84.36	40.02	127.38	16.18
3	Vastus lateralis	F	78	Arteriosclerotis heart disease. Mild diabetic mellitus. Fracture of femur.	80.29	71.17	49.42	120.59	3.88
4	Rectus abdominis	F	47	Rheumatic heart disease. (MS, MI, AS AI) with congestive heart failure.	80.40	41.88	78.94	120.82	0.01
5	Vastus lateralis	F	81	Arteriosclerosis, Parkinsonian, muscle atrophy and bilateral flexion and adduction. Albuminuria.	79.72	79.56	41.35	120.88	2.13
6	Vastus lateralis	F	20	Uremia due chronic glomerulonephritis (Serum K. 7.5 mEq/L)	79.59	91.46	32.67	124.13	0.55
7	Rectus abdominis	M	37	Chronic renal failure. Small intestine perforation (Serum K 4.2, Na 134 mEq/L BUN. 207 mg/100 ml)	80.62	77.31	48.34	125.65	2.75
8	Rectus abdominis	F	65	Esophagitis (stricture of esophagus) Nasal feeding. Hiatus hernia	81.72	62.89	56.76	119.65	2.75

TABLE 15 (B)

DATA OF MUSCLE BIOPSY FROM KIDNEY, HEART OR MUSCLE ATROPHIC PATIENTS

Case No.	Muscle	Sex	Age	Disease and Clinical Signs	Water ml/100g FFDW	K mEq/kg FFDW	Na mEq/kg FFDW	K+Na mEq/kg FFDW
1	Tensor fasciae	M	43	Spinal cord injury in 1958. Muscle atrophy, quadriplegia. Fracture of femur	383.79	315.63	303.43	619.01
2	Gluteus medius	M	57	Fracture of femur in 1963. Disuse muscle atrophy, marked wasting of the thigh musculature	414.14	433.73	221.18	654.91
3	Vastus lateralis	F	78	Arteriosclerotic heart disease. Mild diabetic mellitus. Fracture of femur	407.36	361.09	250.74	611.82
4	Rectus abdominis	F	47	Rheumatic heart disease. (MS, MI, AS AI) with congestive heart failure.	410.20	213.67	402.76	616.43
5	Vastus lateralis	F	81	Arteriosclerosis Parkinsonian, muscle atrophy and bilateral flexion and adduction. Albuminuria.	393.10	392.31	203.90	596.06
6	Vastus lateralis	F	20	Uremia due to glomerulonephritis (Serum K 7.5 mEq/L)	389.96	448.11	160.07	608.18
7	Rectus abdominis	M	37	Chronic renal failure. Small intestine perforation (Serum K 4.2, Na 134 mEq/L BUN. 207 mg/100 ml)	416.00	398.92	249.43	648.35
8	Rectus abdominis	F	65	Esophagitis (stricture of esophagus). Nasal feeding. Hiatus hernia.	447.05	344.04	310.50	654.54

TABLE 16 (A)

RESULTS OF PAIRED OBSERVATIONS ANALYSIS BY t-TEST FOR 124

DUPLICATE SAMPLES

Group	N	Sample size g WW Mean±S.D.	Water ml/100g FFWW Mean	t	P	K mEq/kg FFWW Mean	t	P	Na mEq/kg FFWW Mean	t	P
A	124	0.16±0.04	77.91			89.61			35.95		
				-1.60	N.S.		0.20	N.S.		-1.22	N.S.
B	124	0.14±0.04	77.97			89.43			36.43		

TABLE 16 (B)

RESULTS OF PAIRED OBSERVATIONS ANALYSIS BY t-TEST FOR 124

DUPLICATE SAMPLES

Group	N	Sample size g WW Mean± S.D.	Water ml/100g FFDW Mean	t	P	K mEq/kg FFDW Mean	t	P	Na mEq/kg FFDW Mean	t	P
A	124	0.16±0.04	352.69			405.66			162.74		
				-1.62	N.S.		-0.23	N.S.		-1.88	N.S.
B	124	0.14±0.04	353.93			405.94			165.36		

muscle. They found no significant difference in 10 slices of muscle weighting approximately 10 mg each from 10 slices weighing approximately 100 mg each. In their study of duplicate slices of muscle obtained at operation, however, they reported that the value for potassium on analysis of the small slices (8 - 14 mg) was slightly less than that of larger slices (40 - 50 mg) when the values of small slices were expressed as a percentage of the larger slices. Another check was made by the same authors and it showed that the values for sodium of the 10 mg slices obtained at autopsy were significantly lower than those of the 100 mg slices. Flear suggests that the difference resulted from the failure of obtaining biopsies from an immediately adjacent location. The variation in sodium and potassium in different sample size had been explained by Johnston(36). He postulated that because of the proportionally larger surface area of small samples they are therefore contaminated by excess surface extracellular fluid at the time of biopsy. The surface contamination may be inadequately removed by methods commonly employed for blotting muscle specimens. In the present study in this paper, a trend of a slightly higher water and sodium content in the group of smaller slices of duplicate samples is noted; however, the variation due to different sample size in this study is so small that it is not significant.

In this study it was noted that different muscle groups yielded different potassium and sodium content. This was noted to be true if separate muscles were used for biopsy from the same subject. During the course of this study, Flear et al. in 1965 (24) reported an analysis of muscle composition and its relationship to sample sites. In general, the results reported are found to be in agreement with those

reported in this study. A negative correlation between muscle content of sodium and potassium as shown in Figures 1 (A) and 1 (B) is observed in the 131 adult muscle biopsies when expressed with FFWW or FFDW as the basic reference. This result is in good agreement with Flear's report. However, in the study of 17 children muscle specimens, the negative correlation between sodium and potassium as shown in Figure 3 (A) is demonstrated only when reported with FFWW and not when reported as FFDW as shown in Figure 3 (B). This difference is due to the great variation of water content among those children's muscles. At terms of FFDW, the water content in the muscle is positively correlated with the sum of sodium plus potassium as shown in Figures 2 (B) and 4 (B). This correlation being that the higher the water content in the muscle, the greater the sum of sodium plus potassium may also be found in the study of different muscle composition in relation to the aging process, body growth and sex and in the patients complicated with the muscle wasting, cardiac or renal diseases. Water content variation from 73.0 to 77.6 ml/100g wet weight (Index Table 1) was noted to be high in Flear's series (24) whereas in this study variation of only 77.0 to 79.2 ml/100g FFWW was found. This discrepancy might be due to the use of different reference bases or different analytical method. For instance, the study reported by Flear employed micro method (23) and muscle masses of 10 mg or more wet weight. Therefore, Flear's study employed one-tenth or less sample weight in contrast to the biopsy specimens employed in our study. Although loss of muscle weight due to evaporation is approximately linear with time, the weight loss is not proportional to sample size (36). A small sample of 54 mg may lose more water than a larger sample of 250 mg over the same period of time. The loss due to evaporation in proportion

to the original sample of weight may be larger in the small muscle biopsy samples. Because of this, a great variation of muscle composition in the very small sample may be observed due only to a small error in the estimation. For these reasons, the method of Litchfield and Gaddie employing larger muscle masses was thought to be preferable.

As early as 1957 Holliday et al. (33) reported a differing muscle electrolyte composition if multiple biopsy sites in one subject were studied. Holliday reported difference in muscle potassium content from two sites of the same animal when expressed in terms of fat free dry solid but noted that the difference was not present if by calculating its chloride space, the potassium was expressed in terms of intracellular potassium concentration. It was suggested that this discrepancy was related to differences in connective tissue or extracellular solid in samples from different muscles. Drahota in 1960 (17) also noted differing potassium content of varying muscles from a group of rats, and these differences in potassium content of muscles are of post-natal origin. This author suggested that the difference in intracellular potassium concentration was related to the functional activity of the muscle in that the potassium concentration was lower in the socalled red muscle or mostly red muscle, such as the heart and diaphram of the rat, compared to white muscle, such as m. tibialis anterior of rat. The socalled red muscle appeared to possess an increased content of extracellular sodium related to the greater blood supply in these muscles. The classification of muscle fibre is based on the amount of sarcoplasm and the number of organelles, especially mitochondria, contained in the sarcoplasm. Red fibre generally has a larger amount of sarcoplasm, more nuclei which are more deeply situated, more mitochondria and myoglobin than white fibre (6).

In most both types are mixed with fibre of an intermediate type. Red muscles are adapted for long, slow, posture maintaining contractions, and the white muscles are specialized for fine skilled movement (28). A remarkable recent discovery is that a soleus can be converted from the state of a red muscle to that of a white muscle if it is denervated and and then allowed to be re-innervated by a nerve which originally supplied a white muscle (34). Also gastrocnemius can be made a red muscle if it is denerved and re-innervated by the nerve to soleus (34). In the animals the proportion of red fibres generally increases in deeper muscles next to the bone (9). Wijhe et al. in 1964 (68) noted that human muscle from various anatomical sites differed in their red to white fibre ratios. The application of histo-chemical techniques to the study of normal mammalian skeletal muscle has shown that it is not usually a homogeneous tissue. It contains "red fibres which are rich in dehydrogenase and lipids but poor in glycolytic enzyme and glycogen; white fibres poor in dehydrogenase and lipids but rich in glycolytic enzymes and glycogen"(7). The glycolytic step involving dephosphorylation of phosphoenolpyruvate to pyruvate (P.E.P.A. + A.D.P. $\rightleftharpoons$ Pyruvate + A.T.P.) is mediated by pyruvic kinase. It has been recognized for some time that potassium ions participate in this reaction. The indispensability of K ion for the activity of the pyruvic kinase has been demonstrated by Kachmar et al. in 1953 (37). These differences in enzyme activity (7, 10, 68) may indicate that red muscles have a differing metabolic system from white muscles. In the tables 7 (A) and 7 (B) of this paper, the highest sodium and the lowest potassium contents are found in the intercostal muscles. These deep chest muscles are constantly and automatically functioning day and night. These facts are conducive

to the thought that the differences in sodium and potassium content among muscle groups are related to proportion of red and white fibres in the muscles, their functional activity, metabolic pathway and nervous regualtion of the muscle.

(B) Variation in Water, Potassium and Sodium Content of Skeletal Muscle in Relationship to Body Growth

The results of 17 muscle biopsies obtained from children demonstrated lower potassium and higher water and sodium content when compared to those from adult subjects. A tendency for progressive fall in water and sodium content of the muscle with development, associated with a progressive rise in potassium content is noted. Only limited data on children muscle electrolyte content was discovered by a survey of the literature. Metcoff et al. in 1960 (46) in a study of the metabolic sequences of muscle metabolism in Kwashiorkordisease, analyzed 14 muscle biopsies ranging in age form 1 3/12 to 4 10/12 in years. These samples were also obtained during the course of surgical procedures. Dickerson et al. in 1960 (16) studied the chemical composition of muscle during development but their muscle specimens were obtained during autopsy examination. The data of these two series are tabulated in Table 17 (A) and compared with results in this study.

The results reported in this study are very similar to those reported by both Dickerson and Metcoff. Some variations are noted and may be related to different muscle specimens, sites and differences in average ages.

Differing muscle composition is noted not only in newborn but also in children in the prepubertal group in this study. In Litchfield

TABLE 17 (A)

MUSCLE VALUES IN CHILDREN, PUBLISHED BY VARIOUS AUTHORS

Author	Dickerson Series (1960)			Metcoff Series (1960)	Present Series (1966)	
Material	Post-mortem			Living tissue	Living tissue	
No. of cases	4	3	3	14	17	89 Subjects 131 Samples
Age	Newborn babies	4-7 months babies	Adults	1 3/12 to 4 10/12 years old	7 weeks to 13 years old	14 to 86 years old
Site	Thigh	Thigh	Thigh	?	see Table 10(A)	see Table 7(A)
Water ml/ 100g FFWW	80.4	78.5	76.0	*78.3	78.9	77.8
K mEq/ kg FFWW	57.7	89.5	102.0	*80.8	75.6	89.8
Na mEq/ kg FFWW	60.1	50.1	30.0	*48.5	51.2	35.9

*

The original data of Metcoff's series were expressed on the basis of 100g dry fat free solid. The data of water, K and Na are 359 g/100g DFFS, 37.1 m Mole/100g DFFS and 22.3 m mole/100g DFFS respectively.

and Gaddie's series in 1958 (39), one muscle biopsy was taken from the brachial radialis muscle of a 12 year old boy and it is noted that this showed a lower potassium, higher sodium and water content when compared to that of the adults in their series. (By arranging their patients in order of age, the child's water value was noted to stand third highest of total patents studied. The potassium of the 12 year old boy was second lowest and sodium was the highest among total patients studied.) Although different muscle sites may have been employed, the differences might also be explained by the younger age of the patient studied.

The differences of water and electrolyte composition in muscle when children are compared to adults has been explained as due to an associated decrease in extracellular fluid and an increase in the "Cell mass" with age. Employing chloride spaces, Dickerson et al. in 1960 (16) showed those differences in partition of tissue water, potassium and sodium in human muscles. An increase in "cell mass" has been repeatedly confirmed by histological studies. MacCallum in 1898 (44) studied the sartorius muscle in man and found that the full number of cells in the muscle is reached by approximately six months of fetal life. Henceforth, the increase in size of the muscle is presumed to result from increase in the size of individual muscle cells. The numbers of fibre remains approximately constant. Dickerson et al. in 1960 (16) studied transverse sections of human mid-quadriceps muscle of the fetus, newborn and adults and reported histological appearance of muscles from different age groups. The fetal muscle cell is smaller and more widely separated by extracellular material when compared with that of adult muscle. This is felt to indicate that the extracellular phase of skeletal

muscle will gradually be occupied by the increased size of muscle cells with development and the extracellular fluid would thereby be eliminated. The decrease in extracellular fluid volume in the body with grwoth is also demonstrated by Friis-Hansen in 1957 (26). He reported that extracellular component, determined by thiosulfate method exhibits a gradual decrease from around 45% of total body weight at birth to 18% of total body weight at puberty.

(C) Variation in Water, Potassium and Sodium Content of Skeletal Muscle in Relationship to the Aging Process.

Analysis of 18 muscle biopsies removed from the female pectoralis major with an age range of 18 to 86 years was examined. It was found that water and sodium content and the sum of sodium plus potassium in the muscles of the older age group (average age of 72) were higher than those of young age groups (average age of 44) when expressed with FFDW. An increase in water content in the muscle of older age group was also found when expressed with FFWW. This study may partly confirm a paper published by Simms and Stolman of 1931 (60) who studied human muscle obtained from autopsies following trauma and noted that in six muscles taken from an older age group (average age of 75) an increase in water, chloride and sodium and a decrease in potassium, magnesium, phosphorus and nitrogen were present, when compared with a younger age group's (average age of 55). In the comparison of published data of other investigators (Appendix Table 2) the highest muscle values for water and sodium were noted in the series of Barnes et al. (5) which the average age was 66 years, and was highest among all series and the muscle value for potassium in the series of Barnes et al. was no appreciable difference as compared to others.

In the relevant literature, many papers have been published on the composition of skeletal muscle in the aging animal. Lowry et al.in 1941 (43) reported that the water and chloride concentration of skeletal muscle (FFWW)increases while the potassium content declines in the senile rat. Holliday et al. in 1957 (33) also reported that lower intracellular potassium with reciprocal increase in intracellular muscle sodium was noted in the aging rat. Yiengst et al. in 1959 (69) analyzed 40 MacCallum rat muscles and found there is a small but significant increase of the total muscle water content in old male rats and a trend of an increase in that of old female rats. (Male rat P = 0.001, Female rat P = 0.07). A significant decrease in the intracellular water and intracellular component nitrogen, potassium and phosphorus in the old animal is noted when expressed with FFWW. They interpreted that uniform loss of the intracellular components suggests a loss of muscle fibre during aging. Andrew et al. in 1959 (3) had histologically studied in the muscle of the same rats that were analyzed chemically by Yiengst et al.. Andrew's results are in agreement with Yiengst's study. The histological finding demonstrated that in old rats some portions of muscle (a tissue composed of irreversible post-mitotic unit) no longer occupied by striated fibre filled in either by fibrous connective tissue or mature type of adipose tissue and the mast cell were more abundant in the muscle. The sarcoplasm itself showed marked degenerative changes with fragmentation of fibres and decrease in size and disappearance of fragment in old rats. It indicates a decrease in size of the individual muscle fibre with a relative increase in volume of space between the fibre in which is depots of extracellular fluid. In this fluid water and sodium concentration are

higher than in the intracellular phase. Allen et al. in 1960 (1,2) in their study on human body compositions which were measured by gamma ray whole body counting of the naturally occurring k^{40} isotope reported that the ratio of body potassium to dehydrated defatted protoplasmic mass of body decreases with increasing age and is lower in the female.

In the study reported in this paper an increase in muscle water and sodium content in the older age group may be explained by the loss of muscle fibre and a relative increase in extracellular fluid with age. The result of no significant decrease in the muscle potassium content in the older age group could be interpreted that the negative change in muscle potassium with age is much less than the positive change in sodium in aging process. It is also possible to accumulate fluid in muscle due to some causes other than muscle fibre loss.

Hilton et al. in 1955 (31) found that the degree of metabolic acidosis induced by prolonged administration of ammonium chloride was of greater severity in the geriatric group than in the young group. Moore in 1931 (50) demonstrated that the number of glomeruli in normal human kidney decreases progressively with age. Shock et al. in 1958 (58) reported the reduction of kidney function with age but they failed to demonstrate any systematic changes either in plasma volume or in total extracellular fluid volume (59). According to Shock's studies, the reduction of kidney function is not likely an important factor causing the change of muscle composition in old age groups; however, this may contribute to it.

Swyer in 1958 (64) studied the hormonal aspects of water and electrolyte metabolism in relation to age and sex but no conclusion was made.

Keys et al. in 1957 (38) studied many groups of adults who were different in occupation and found that the more "active" man tends to be leaner while the more "sedentary" individual is more frequently classified as fat. Pařízková in 1963 (57) confirmed that the influence of intensity of physical activity on body composition is evident in childhood, adulthood and old age and causes an increase in lean body mass (a further increase in size of muscle cell) at the expense of fat. If the young adult always does heavier work than the older one, the difference of physical work could be one of the factors causing variation in muscle composition between young and old subject.

A significant increase in neutral fat in the older group as compared to the young age group was found in this study. The results are shown in Table 13 (A). This result is supported by the finding of Friis-Hansen in 1964 (27) who reported his study on the microphotographs of cross section from 74 year old woman demonstrated that of partly fatty degeneration of the muscle cells.

Parker et al. in 1958 (55) compared 7 normal males and 7 normal females of average ages 75 and 68 respectively, with young adults and found a relative decrease in intracellular water and total body water and a relative increase in total body solid in old age groups but no change in extracellular water in value related to body weight. These changes may also indicate an accumulation of body fat and loss of muscle cell with aging process.

(D) Variation in Water, Potassium and Sodium Content of Skeletal Muscle in Relationship to Sex

In this study the amount of water and the sum of sodium plus potassium are relatively greater in the muscle of the female than that

of the male when expressed with FFDW. This result again shows that the higher the water content in the muscle, the greater the sum of sodium plus potassium in terms of FFDW. A higher water content in female muscle when measured with FFWW is also noted. In review of the relevant literature, no paper on discussion in the water or electrolyte contents in human muscle in relation to sex is found. Bergström in 1962 (8) had studied a large series of human muscle biopsy obtained from 33 males and 13 females at the site of quadriceps femoris but he does not discuss the variation between the sexes. A statistical study of the series in his appendix table (muscle values in normal subjects results obtained from individual muscle specimens) shows that the water and the sum of sodium plus potassium in the female muscle are significantly higher than that in male when expressed with FFDW. The muscle water content difference between female and male was up to 1 ml per 100g of fat free wet weight which is in good agreement with present results. It is well known that there is a higher relative content of total body water, intracellular water and potassium and lower extracellular water in male than in female (1, 2, 40, 49, 55). These differences were interpreted by Parker et al. in 1958 (55), as the result of a higher development of tissue richer in intracellular material and relatively poor in extracellular phase such as muscle tissue in male and a higher relative amount of fat in female. This interpretation of these differences due to muscle development is supported by Suárez and Marquesán in 1957 (62) who found a close correlation between the intracellular water (measured as the difference between total water by antipyrine space and the extracellular water by thiocyanate space) and the radiographically determined cross sectional muscle area of the leg (63). Because of higher development of the muscle in male the extracellular phase

of muscle could be diminished and occupied by the increasing size of muscle cell. It is believed that the ratio of extracellular fluid to intracellular fluid of muscle is higher in the fmeale than that in the male. This assumption is supported by present results that there is a relatively higher water content in the female than in male when expressed with FFWW or FFDW; however, no significant difference in muscle sodium or potassium content between the sexes is noted. It could not be interpreted just by difference in muscle development.

Many women gain in body weight before menstruation (12, 64). Premenstrual weight gain could be a contributing factor of retention of water and perhaps sodium in the female but it is difficult to explain that of the age group over 55 years in our series, as there is not a great difference of urinary estrogen between female and male at the ages of 50 to 70 years. Furthermore, the output of urinary estrogen is somewhat lower in the female than in the male after the age of 70 years (56). Szimai in 1962 (65) reported that the androgen and estrogen can influence the anatomic structure and chemical composition of different animal tissue in distinctly different ways. Could endocrinological difference be responsible for different human muscle composition in relation to sex? It still remains to be further studied. Ljunggren et al. in 1957 (41) reported that extracellular water is significantly greater in relation to body weight in the male than in the female but he also noted that the female has more body fat than the male. (The values of body fat are 20 - 30% of body weight in normal woman and 10 - 20% for male of normal body weight). The fat tissue contains very little water, only about 10 - 20%. Any increase in the fat tissue of body will therefore cause a decrease

of relative water content. An increase in extracellular water in relation to body weight does not indicate that extracellular water in the muscle is larger in the male than in the female.

At the present time it is impossible to give a satisfactory explanation for the difference of muscle water content between male and female.

(E) Muscle Biopsies from Patients with Muscle Wasting, Cardiac or Renal Disease

In this study (Table 15) three muscle biopsies were obtained from disturbance of muscle function or wasting diseases as Case 1 (spinal cord injury, quadriplegia since 8 years ago), Case 2 (disuse muscle atrophy due to femur fracture in 1963) and Case 8 (stricture of esophagus - muscle wasting, progressive loss of weight for 3 years, nasal feeding and semi-starvation). Two biopsies were taken from heart diseases as Case 3 (arteriosclerotic heart disease) and Case 4 (chronic valvular disease) and another three muscle biopsies were taken from renal complications as Case 5 (arteriosclerosis, muscle atrophy, urinary tract infection and albuminuria), Case 6 (uremia with hyperkalemia, serum K:7.5 mEq/l) and Case 7 (uremia with small intestinal perforation). All muscle biopsies from these patients show higher water and sodium content with lower potassium in muscle (except Case 6) when compared to the muscle values of normal adults in this paper. Moore et al. in 1963 (49) explained that the changes of body composition in those patients with chronic disease are loss of body cell mass, gain in extracellular volume and oxidation of fat. They described that in the patient with wasting disease, the body cell mass is undergoing wasting with no change in the relative content of extracellular fluid bathing the cell. In

the chronic cardiac patient it is accumulation of of fluid due to impaired excretion of water and salt on one hand and loss of cell mass and fat due to circulatory starvation of the tissue on the other hand, (but the loss of body cell mass is minor or absent in heart disease secondary to primary pulmonary disease). In the patient with chronic renal failure they postulated that loss of body cell mass is not great so long as oral caloric intake is not compromised, since there is no circulatory defect in delivery of nutriment to the tissue. The composition change is thus due far more to an increase in extracellular components than to a decrease in cell mass. In general, the explanation by Moore et al. is acceptable.

Metcoff et al. in 1960 (46) found not only increase in extracellular fluid but also a swelling of muscle cell with the change in concentration of intracellular ions in the children with severe Kwashiorkor. The change of composition in muscle atrophy as in Case 2 may be completely interpreted by a shrinking cellular mass and relative accumulation of the extracellular fluid as body wasting occurs through the disuse but in Case 8 (muscle wasting due to malnutrition) a change in the intracellular component may take place in addition to an increase in the extracellular fluid and loss of the cell mass.

The results that demonstrate a deficit of potassium and overhydration in the muscle of patients with congestive heart failure is similar to that published by other authors (13, 20). Moore et al. (49) reported that chronic acquired adult valvular heart disease wherein the composition disorder is maximal, hypotonicity regularly occurs, a very low ratio of total exchangeable sodium plus potassium to total body water is observed. Results of analysis in cardiac patients in this paper also showed that the

sum of sodium plus potassium in the muscle is lower when expressed with kg FFWW as basic reference and compared with mean value of normal adults or children. On the contrary, when expressed with kg FFDW as basic reference the sum of sodium plus potassium is higher in the muscle of patients with cardiac disease. It is not surprising to find those results because total dry solid per unit of wet muscle tissue in the patient with cardiac disease is less than that in the normal adult.

The finding in Case 6 (uremia with hyperkalemia, serum K: 7.5 mEq/l) showing no increase in potassium content in the muscle is quite interesting. Bergström in 1962 (8) interpreted that the capacity to accumulate potassium within the muscle cell is very limited and this is probably due to a limitation of the number of nondiffusible intracellular anions available.

For determination of acute water and salt retention by using the value of muscle biopsy in the patients with chronic diseases, the increase in water content due to the chronic loss of cell mass must be taken into account. The Muldowney's idea of interpretation of clinical alteration in body water and electrolytes by using the values of muscle biopsy is very good, but before application to clinical use the normal data with correction for age, sex and muscle site differences must be available.

V. SUMMARY

1. Skeletal muscle biopsies obtained from 89 adults (131 samples), 17 children (17 samples) and 8 patients with disturbance of cardiac, renal or muscle function, were analyzed for water, sodium and potassium content using the chemical and flame photometric method of Litchfield and Gaddie.

2. The analytical methods employed in this project have been studied and shown to be reliable for purposes of the present study.
3. Muscle sample sizes ranged from 0.14 to 0.16g wet weight. 124 duplicate samples were analyzed. Duplicate samples analyses of heavy weight were compared with lighter and smaller sample size. No significant difference in sodium, potassium and water content was discovered.
4. The mean values of muscle composition are tabulated in the Tables 7 (A) and 7 (B) for adults and Tables 10 (A) and 10 (B) for children. The results from normal subjects are essentially in agreement with results published by previous investigators.
5. Different muscle groups vary in sodium and potassium content, and these variations are noted in samples from different muscles obtained from the same subject.
6. Negative correlation between sodium and potassium content is demonstrated when expressed with fat free wet weight (FFWW) or fat free dry weight (FFDW) as the basic reference. However, the negative correlation may not be present in the muscle samples with a great variation of water content in terms of FFDW as shown in Figure 3 (B). A positive correlation between the muscle content of water and the sum of sodium plus potassium is observed when expressed with FFDW.
7. A significant difference in potassium, sodium and water content in the muscle of 17 children as compared with adults is noted, the potassium being lower and the water and sodium being higher in the children.
8. Muscle biopsies were obtained from the sites of the pectoralis major of 18 females, age 18 to 86 years. It was found that water

and sodium content in the muscles of the older age group (average age of 72 years) was higher than those of the younger age group (average age of 44 years) when expressed with FFDW and only an increase in water content in the muscles of the older group is noted when expressed with FFWW.

9. A significant difference in water content of female muscle from that in male muscle is found, being higher in the female.
10. Muscle edema in patients with muscle wasting, cardiac or renal disease and muscle biopsy in its clinical application were discussed.

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VII. APPENDIX (TABLES)

APPENDIX TABLE 1 (A)

COMPOSITION OF VARIOUS MUSCLE IN 43 CONTROL SUBJECTS PUBLISHED BY C.T.G. FLEAR ET AL IN 1965 (24)

MUSCLE	Water ml/100g			*Na mEq			*K mEq		
	Mean	s.e.	No.	Mean	s.e.	No.	Mean	s.e.	No.
Rectus abdominis	77.1	0.53	9	28.5	1.50	9	86.7	2.43	9
Pectoralis major	76.6	0.37	12	27.5	1.30	12	93.1	1.87	12
Serratus anterior	76.3	0.79	9	30.4	2.15	9	89.4	1.77	9
Latissimus dorsi	75.9	0.88	8	35.5	2.75	8	88.8	2.38	8
Intercostal	73.8	1.56	7	42.2	4.03	7	74.9	4.10	7
Trapezius	77.5	0.53	5	30.9	2.15	5	92.8	3.29	5
Rhomboid	77.6	--	2	35.0	--	2	94.0	--	2
Erector spinae	74.6	--	1	26.0	--	1	90.0	--	1
Diaphragm	75.7	--	1	32.3	--	1	88.8	--	1
Deltoid	73.0	1.33	7	49.3	3.03	7	68.4	3.92	7
Gastrocnemius	75.9	1.20	4	35.2	6.44	4	83.3	6.77	4

No.: Number of subjects: the mean value taken from each individual is based upon three values, one from each site sampled.

* The amounts of electrolytes are those contained in 1 kg. of wet tissue.

APPENDIX TABLE 2 (A)

MUSCLE VALUES IN NORMAL SUBJECTS PUBLISHED BY VARIOUS AUTHORS

AUTHOR	N	Av Ag	Mean±S.D. per kg FFWW Water ml	K mEq	Na mEq	Biopsy Site	Remarks
Mudge et al., 1949 (51)	3	47	793	85.6	39.7	R	GA
Eliel et al., 1951 (19)	5		791±10	83.7	29.5	P	
Mokotoff et al., 1952 (47)	4	39	782	102.5	25.3	G	SA
Baldwin et al., 1952 (4)	15			94.6±11.7			GA.F.
Talso et al., 1953 (66)	16	47	776.6±6.4	94.0± 5.9	33.7±6.4	R.Q.L.	GA.SA.LA.
Cort et al. 1954 (13)			779	96.0	29.2		
Wilson, 1955 (67)	13		775.2	92.3±7.6	40.6±6.0	R.O.	GA
Barnes et al., 1957 (5)	10	66	803±16	91.3±8.3	43.6±11.0	Sb.P.Se.Sg.	GA.SA.
Nichols, 1958 (53)	6	59	784±4.9	91.0±7.6	39.8±7.9	R	GA
Litchfield et al., 1958 (39)	23	44	772±6.2	101.8±4.7	31.6±4.2	R.Tr.Q.P.Br.	GA.LA.
Lorenzini, 1959 (42)	25		775.3±6.8	87.1±4.9	29.1±3.7	R	GA
Flear et al., 1960 (21)	18		757±22	80.3±15.3	31.71±8.5	R.D.	GA.F
Bergström 1962 (8)	46	31	774.5±9.7	100.9±5.4	26.0±5.5	Q	LA.AA.
Flear et al., 1965 (24)	43		758.6	89.0	33.9	See Ap. Table 1	GA.F.
Palmier et al., 1965 (54)	20		787	78.8	30.46	P	GA
Present Author	89	44	778±7.8	89.8±7.7	35.9±8.8	See Table 7	GA

Symbol:
- Br: Brachio-radialis
- D: Deltoid
- G: Gastrocnemius
- L: Latissimus dorsi
- O: Other sites
- Av ag: average age
- AA: Activation analysis
- LA: Local anesthesia
- F: Fat not extracted
- P: Pectoralis
- Q: Quadr. fem.
- R: Rectus abd.
- Sb: Semitendinous & biceps fem.
- Sg: Soleus and gastrocnemius
- St: Sternocleidomastoideus
- Tr: Transverse or obliq. abd.
- N: Number of subjects
- S.D.: Standard Deviation
- GA: General anethesia
- SA: Spinal anesthesia

Iseri's series (Autopsy material): N=11 Site : pectoralis major

Water = 772±19 ml, K = 94.2±26.5 mEq and Na = 40.6±12.6 mEq (mean±S.D.)/ kg of FFWW.

APPENDIX TABLES 3 (B)

BERGSTRÖM'S SERIES: MUSCLE VALUES IN NORMAL SUBJECTS

RESULTS OBTAINED FROM INDIVIDUAL MUSCLE SPECIEMNES (8)

			Water g per		K mEq per		Na mEq per	
No.	Sex	Age	100g Fat Free Solid		100g Fat Free Solid		100g Fat Free Solid	
1	M	35	337	331	43.2	42.7	10.1	8.5
2	M	21	339	327	42.1	44.5	12.4	8.4
3	M	26	348	333	44.3	43.1	13.2	11.3
4	M	34	326	322	47.8	39.6	8.1	19.6
5	M	25	348	329	43.2	44.0	13.5	10.2
6	M	25	343	333	43.6	40.5	12.5	16.3
7	M	35	337	324	44.0	44.1	12.3	11.1
8	M	49	333	333	44.9	47.6	9.5	7.1
9	M	41	350	346	46.3	40.1	13.3	18.6
10	M	25	329	331	41.6	--	12.4	15.1
11	M	26	369	367	46.5	46.4	8.9	9.9
12	M	24	337	--	48.1	--	11.4	10.4
13	M	41	355	350	46.9	48.5	10.6	11.2
14	M	40	361	--	44.3	45.1	16.9	13.5
15	M	33	339	352	44.0	43.4	13.3	16.0
16	M	42	357	355	46.1	44.2	12.4	13.7
17	M	19	344	--	42.1	--	11.5	16.9
18	M	31	376*	381*	--	--	12.8	11.0
19	M	24	313	326	42.2	40.0	9.6	13.2
20	M	19	312	298	44.8	45.2	7.4	8.1
21	M	39	326	331	45.9	44.0	8.0	8.7
22	F	33	329	329	42.3	44.0	12.8	11.4

*

Case 18: Water content determined on other samples than those used for electrolyte determination.

No.	Sex	Age	Water g per 100g F	Free Solid	K mEq per 100g Fat	Free Solid	Na mEq per 100g Fat	Free Solid
23	F	30	355	337	44.6	47.1	12.1	8.3
24	F	28	365	--	--	--	10.4	12.5
25	F	23	372	363	--	--	13.5	13.0
26	F	39	363	369	43.5	45.0	11.9	13.2
27	F	38	329	341	43.0	43.3	12.9	13.1
28	F	28	367	--	44.4	--	19.3	16.0
29	M	59	361	374	--	--	11.2	16.6
30	M	51	348	363	--	--	11.5	14.6
31	M	27	322	--	47.0	--	11.1	--
32	M	29	322	--	44.1	--	9.6	--
33	M	27	344	--	46.0	--	8.3	--
34	M	24	341	--	43.8	--	11.5	--
35	M	27	324	--	42.9	--	8.1	--
36	M	28	333	--	45.4	--	9.7	--
37	M	26	333	--	--	--	9.1	--
38	M	35	355	--	--	--	11.6	--
39	M	22	341	--	--	--	10.5	--
40	M	22	329	--	--	--	11.1	--
41	F	23	346	--	--	--	10.5	--
42	F	24	388	--	45.9	--	13.2	--
43	F	29	398	--	46.6	--	16.0	--
44	F	38	337	--	44.8	--	14.1	--
45	F	35	348	--	47.7	--	10.4	--
46	F	24	376	--	--	--	9.8	--

APPENDIX TABLE 4 (A)

MUSCLE VALUES IN 44 SIBJECTS

RESULTS OBTAINED FROM 22 FEMALE AND 22 MALE MUSCLE SPECIMENS

No.	Sex	Age	Sites	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	Fat g /100g WW
1	F	48	Rectus abdominis	76.75	75.30	57.60	1.75
2	F	41	" "	77.76	84.10	32.33	4.51
3	F	43	" " "	77.66	97.07	28.17	1.33
4	F	40	" "	76.70	89.44	34.65	2.25
5	F	44	" "	78.61	84.99	42.04	4.85
6	F	47	" "	78.24	95.18	32.97	3.59
7	F	42	" "	77.24	93.59	40.09	0.21
1	M	51	" "	76.72	88.21	40.00	2.69
2	M	32	" "	76.70	93.21	28.36	0.23
3	M	55	" "	76.63	90.10	25.26	0.96
1	F	20	Vastus lateralis	79.37	86.42	38.06	1.09
2	F	37	" "	77.75	88.51	40.39	1.66
1	M	20	" "	78.86	84.70	37.49	3.17
2	M	18	" "	75.69	93.66	32.14	0.69
3	M	40	" "	77.74	93.31	30.58	6.10
4	M	19	" "	77.57	98.32	26.83	0.33
1	F	47	Latissimus dorsi	78.34	89.50	30.80	1.10
2	F	47	" "	78.03	95.37	39.84	3.03
3	F	54	" "	79.01	85.90	45.69	6.17
1	M	56	" "	77.15	93.46	31.42	0.59
2	M	61	" "	77.46	84.29	33.46	1.31

No.	Sex	Age	Sites	Water ml/100g FFWW	K mEq/kg FFWW	Na mEq/kg FFWW	Fat g /100g WW
3	M	29	Latissimus dorsi	76.47	94.23	32.58	1.76
1	F	20	Vastus intermedius	79.63	73.08	50.28	0.47
2	F	21	" "	77.92	95.69	25.35	0.06
1	M	32	" "	78.36	89.40	39.48	1.08
2	M	18	" "	76.56	88.49	38.33	0.87
1	F	38	Erector spinae	78.28	79.90	42.09	5.23
2	F	40	" "	78.27	84.88	34.52	1.94
1	M	52	" "	77.48	71.83	51.68	0.74
2	M	32	" "	78.63	76.72	42.20	1.76
3	M	27	" "	76.33	87.30	30.24	2.51
4	M	27	" "	76.41	95.52	29.29	1.38
5	M	31	" "	76.58	98.65	27.73	3.38
6	M	49	" "	77.34	96.65	23.59	4.77
1	F	54	Intercostal	78.07	77.78	51.95	3.35
2	F	60	"	78.67	69.42	61.38	3.87
3	F	54	"	78.71	82.35	45.93	3.30
1	M	56	"	77.38	84.14	39.62	6.01
2	M	61	"	77.85	71.64	53.28	3.60
1	F	54	Serratus anterior	77.54	90.59	42.30	1.09
2	F	55	" "	78.80	90.81	29.48	4.59
3	F	54	" "	78.48	93.92	34.58	4.03
1	M	56	" "	77.61	93.64	33.49	2.05
2	M	53	" "	78.41	73.10	52.36	1.94

APPENDIX TABLE 5 (A)

MUSCLE VALUES IN 89 NORMAL ADULTS. RESULTS OBTAINED FROM 131 MUSCLE SPECIMENS

No.	Sex	Age	Biopsy Site	kg FFWW			100 g WW
				Water ml	K mEq	Na mEq	Fat g
1	F	45	Pectoralis major	776.1	99.35	24.01	1.01
2	F	40	" "	772.7	90.11	29.97	0.19
3	F	53	" "	780.4	91.22	28.84	0.45
4	F	36	" "	775.9	95.14	21.09	1.31
5	F	73	" "	780.8	91.53	31.61	2.89
6	F	47	" "	784.7	81.60	45.50	1.17
7	F	45	" "	775.1	99.31	29.46	1.04
8	F	60	" "	791.4	90.61	42.17	3.00
9	F	86	" "	785.4	98.41	36.84	2.98
10	F	56	" "	775.5	99.62	25.88	0.79
11	F	79	" "	775.9	94.37	41.38	8.19
12	F	44	" "	777.9	95.33	31.48	0.69
13	F	74	" "	788.8	92.79	32.25	2.94
14	F	55	" "	786.1	101.70	30.26	1.35
15	F	18	" "	773.0	93.86	35.30	1.00
16	F	70	" "	782.5	86.11	48.96	0.82
17	F	48	" "	782.0	95.06	27.69	0.60
18	F	63	" "	782.9	95.77	24.38	1.71
19	F	40	Pectoralis Minor	771.7	94.00	26.54	0.96
20	F	36	" "	779.2	94.83	21.84	1.65
21	F	47	" "	788.9	82.09	42.98	0.56
22	F	45	" "	772.0	100.86	23.28	1.65
23	F	44	" "	792.6	93.40	32.46	0.69

No.	Sex	Age	Biopsy Site	kg FFWW Water ml	kg FFWW K mEq	kg FFWW Na mEq	100 g WW Fat g
24	F	48	Pectoralis minor	777.1	103.68	19.89	0.03
25	F	53	" "	783.4	92.30	26.45	0.69
26	F	60	" "	790.9	86.84	45.55	2.09
27	F	56	" "	778.5	96.29	36.14	4.79
28	F	55	" "	782.6	103.44	30.27	0.66
29	F	63	" "	783.2	95.55	26.75	1.97
30	F	77	" "	786.9	95.79	28.29	0.81
31	F	73	" "	783.0	93.76	28.63	3.55
32	F	86	" "	786.8	95.95	32.33	1.62
33	F	79	" "	777.2	98.64	30.86	2.93
34	F	74	" "	783.7	100.43	26.75	1.07
35	F	21	Rectus abdominis	773.2	85.36	33.40	3.26
36	F	27	" "	789.8	74.55	50.21	2.06
37	F	32	" "	781.0	88.14	40.20	1.30
38	F	39	" "	771.5	91.59	38.52	1.13
39	F	36	" "	788.4	71.61	60.48	3.86
40	F	39	" "	776.6	92.41	36.05	6.52
41	F	33	" "	784.2	77.18	48.50	1.86
42	F	24	" "	772.9	75.79	47.10	1.63
43	F	37	" "	773.4	91.13	32.24	3.06
44	F	39	" "	777.8	93.41	37.48	2.03
45	F	48	" "	767.5	75.30	57.60	1.75
46	F	41	" "	777.6	84.10	32.33	4.51
47	F	43	" "	776.6	97.07	28.17	1.33
48	F	40	" "	767.0	89.44	34.65	2.25
49	F	44	" "	786.1	84.99	42.04	4.85

No.	Sex	Age	Biopsy Sites	kg FFWW			100 g WW
				Water ml	K mEq	Na mEq	Fat g
50	F	47	Rectus abdominis	782.4	95.18	32.97	3.59
51	F	42	" "	772.4	93.59	40.09	0.21
52	M	51	" "	777.2	88.21	40.00	2.69
53	M	32	" "	767.0	93.21	28.36	0.23
54	M	55	" "	766.3	90.10	25.26	0.96
55	F	20	Vastus Lateralis	793.7	86.42	38.06	1.09
56	M	15	" "	783.5	90.88	33.72	1.17
57	F	49	" "	772.1	97.28	29.85	2.36
58	F	39	" "	777.5	88.51	40.39	1.66
59	M	20	" "	788.6	84.70	37.49	3.17
60	M	18	" "	756.9	93.66	32.14	0.69
61	M	40	" "	777.4	93.31	30.58	6.10
62	M	19	" "	775.7	98.32	26.83	0.33
63	M	70	" "	780.1	86.99	44.58	2.60
64	F	56	" "	788.5	93.01	33.49	2.17
65	F	68	" "	776.2	94.18	27.24	3.26
66	F	57	" "	783.6	91.02	37.88	1.26
67	F	53	" "	776.9	91.33	32.21	0.85
68	F	86	" "	784.7	90.32	31.73	4.88
69	M	32	Vastus intermedius	783.6	89.40	39.48	1.08
70	F	20	" "	796.3	73.08	50.28	0.47
71	F	56	" "	789.4	91.45	35.54	1.62
72	F	21	" "	779.2	95.69	25.35	0.06
73	M	18	" "	765.6	88.49	38.33	0.87
74	M	52	Erector spinae	774.8	71.83	51.68	0.74
75	M	32	" "	786.3	76.72	42.20	1.76

No.	Sex	Age	Biopsy Site	kg FFWW			100 g WW
				Water ml	K mEq	Na mEq	Fat g
76	M	27	Erector spinae	763.3	87.30	30.24	2.51
77	F	38	" "	782.8	79.90	42.09	5.23
78	F	54	" "	778.9	95.01	34.65	3.90
79	M	27	" "	764.1	95.52	29.29	1.38
80	F	53	" "	794.6	74.10	53.06	12.97
81	M	31	" "	765.8	98.65	27.73	3.38
82	M	49	" "	773.4	96.65	23.59	4.77
83	F	40	" "	782.7	84.88	34.52	1.94
84	F	54	Intercostal	780.7	77.78	51.95	3.35
85	M	56	"	773.8	84.14	39.62	6.01
86	M	61	"	778.5	71.64	53.28	3.60
87	F	60	"	786.7	69.42	61.38	3.87
88	F	54	"	787.1	82.35	45.93	3.30
89	M	29	"	766.2	82.66	42.19	4.57
90	F	54	Serratus anterior	775.4	90.59	42.30	1.09
91	F	55	" "	788.0	90.81	29.48	4.59
92	M	56	" "	776.1	93.64	33.49	2.05
93	M	53	" "	784.1	73.10	52.36	1.94
94	F	54	" "	784.8	93.92	34.58	4.03
95	M	29	" "	763.2	96.12	24.08	0.97
96	M	18	Deltoid	770.8	72.50	53.46	0.38
97	M	30	"	764.0	93.17	31.02	0.05
98	M	31	"	768.7	86.01	42.82	2.21
99	M	42	"	776.3	89.70	37.56	3.20
100	M	24	"	771.1	93.78	30.91	0.11

No.	Sex	Age	Biopsy Site	kg FFWW Water	K mEq	Na mEq	100 g WW Fat g
101	F	86	Tensor fasciae latae	791.4	89.87	32.63	14.40
102	M	28	" " "	771.8	97.13	30.70	5.26
103	F	27	" " "	778.7	85.71	43.15	5.20
104	M	31	" " "	767.8	88.94	39.04	4.47
105	M	14	" " "	774.9	86.68	34.61	1.88
106	M	56	Latissimus dorsi	771.5	93.46	31.42	0.59
107	M	61	" "	774.6	84.29	33.46	1.31
108	F	47	" "	783.4	89.50	30.80	1.10
109	F	47	" "	780.3	95.37	39.84	3.03
110	F	54	" "	790.1	85.90	45.69	6.17
111	M	29	" "	764.7	94.23	32.58	1.76
112	M	31	Gluteus medius	768.5	87.36	33.67	3.09
113	F	58	" "	776.9	80.55	41.96	6.74
114	M	39	Gluteus maximus	773.7	79.26	44.78	6.88
115	F	27	" "	788.2	96.33	29.90	6.43
116	M	40	" "	779.5	81.99	44.92	11.53
117	M	57	Vastus medialis	768.1	102.17	30.99	2.04
118	F	15	" "	777.1	89.86	32.87	0.03
119	M	32	Rectus femoris	765.3	89.24	37.50	2.27
120	F	15	" "	774.5	103.88	19.19	0.00
121	F	54	Rhomboid major	780.2	78.78	54.76	1.04
122	M	29	" "	771.9	83.00	44.26	0.59
123	M	64	Adductor longus	791.7	82.93	37.95	2.90
124	M	64	Adductor brevis	791.5	96.26	27.53	3.41
125	M	31	Iliacus	772.3	97.61	30.81	1.42

No.	Sex	Age	Biopsy Site	kg FFWW			100 g WW
				Water ml	K mEq	Na mEq	Fat g
126	M	68	Ext. oblique abd.	770.6	82.54	37.97	9.44
127	M	29	Tibialis posterior	777.1	90.68	32.29	2.27
128	F	60	Serratus post. inf.	781.1	97.49	28.07	5.00
129	F	48	Pyramidalis abd.	781.7	92.54	39.53	2.77
130	M	14	Sartorius	776.5	80.70	41.31	0.07
131	F	15	"	773.8	101.61	23.38	0.03
Mean ± Standard Deviation				778.4±7.8	89.76±7.72	35.88±8.75	2.59±2.46

Average age: 44 Total Samples 131 (Female 85, Male 46).